U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-276 978

Biological Effects of Pesticides on the Dungeness Crab

Oregon State Univ, Newport Marine Science Center

Prepared for

Environmental Research Lab, Gulf Breeze, Fla

1 Sep 77

EPA-600/3-77-131
November 1977

PB 276 978
Ecological Research Series

BIOLOGICAL EFFECTS OF PESTICIDES ON THE DUNGENESS CRAB

UNITED STATES · ENVIRONMENTAL PROTECTION AGENCY ·

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Gulf Breeze, Florida 32561

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-77-131	2.	3. RECIPIENT'S ACCESSION NO. PB276978

4. TITLE AND SUBTITLE Biological Effects of Pesticides on the Dungeness Crab	5. REPORT DATE September 1, 1977 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Richard S. Caldwell	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Oregon State University Department of Fisheries & Wildlife Marine Science Center Newport, Oregon 97365	10. PROGRAM ELEMENT NO. 1EA615 11. CONTRACT/GRANT NO. 68-01-0188
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - Gulf Breeze Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, Florida	13. TYPE OF REPORT AND PERIOD COVERED Final 14. SPONSORING AGENCY CODE EPA/600/4

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The toxicity of nine pesticides to various life history stages of the Dungeness crab, Cancer magister, was examined to establish the most sensitive life stage of the crab, and the highest concentration of each pesticide having no discernible effect on that most sensitive stage during prolonged exposures. The compounds tested were the insecticides carbofuran, chlordane, malathion and methoxychlor; the herbicides 2,4-D, DEF, propanil and trifluralin; and the fungicide captan.

For each pesticide the zoeal stages were found to be the most sensitive in long-term tests, approximately 5 to 10 times and 10 to 100 times more sensitive than juvenile and adult crabs, respectively, and were also affected at lower concentrations than those that affected egg hatching and prezoeal development. The maximum acceptable toxicant concentrations for continuous exposures of C. magister zoeae to each of the nine pesticides are: methoxychlor, 0.005 µg/liter; chlordane, 0.015 µg/liter; malathion, 0.02 µg/liter; carbofuran, 0.05 µg/liter; captan, 2 µg/liter; DEF, 4 µg/liter; trifluralin, 15 µg/liter; propanil, 80 µg/liter; and 2,4-D, 1000 µg/liter.

The toxicity of each of these pesticides to crabs is compared with literature reports of their toxicity to other aquatic species.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS		c. COSATI Field/Group
Toxicity	zoeae	Cancer magister	
Invertebrates	larvae	methoxychlor	
Bioassay	captan	insecticide	
Marine Animals	herbicide	2,4-D	
Pesticides	fungicide	propanil	
Crustaceans	carbofuran	DEF	
	chlordane	trifluralin	
	malathion		

18. DISTRIBUTION STATEMENT release to public	19. SECURITY CLASS *(This Report)* unclassified	21. NO. OF PAGES 143
	20. SECURITY CLASS *(This page)* unclassified	22. PRICE PC-A07/MF-A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

BIOLOGICAL EFFECTS OF PESTICIDES ON THE DUNGENESS CRAB

by

Richard S. Caldwell
Department of Fisheries and Wildlife
Marine Science Center
Oregon State University
Newport, Oregon 97365

Contract No. 68-01-0188

Project Officer

Marlin E. Tagatz
Environmental Research Laboratory
Gulf Breeze, Florida 32561

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE, FLORIDA 32561

DISCLAIMER

This report has been reviewed and approved for publication by the Environmental Research Laboratory, Gulf Breeze, U.S. Environmental Protection Agency (EPA). Approval does not signify that the contents necessarily relfect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

.the effects of toxic organic pollutants on individual species and communities or organisms;

.the effects of toxic organics on ecosystem processes and components;

.the significance of chemical carcinogens in the estuarine and marine environments.

This report describes the toxicity of nine pesticides to various life history stages of the Dungeness crab. The data obtained for each pesticide, which identify the crab's most sensitive stage and the highest concentration having no discernible effect on that most sensitive stage during prolonged exposures, will be useful in establishing saltwater quality criteria.

Thomas W. Duke
Director
Environmental Research Laboratory

ABSTRACT

The toxicity of nine pesticides to various life history stages of the Dungeness crab, Cancer magister, was examined to establish the most sensitive life stage of the crab, and the highest concentration of each pesticide having no discernible effect on that most sensitive stage during prolonged exposures. The compounds tested were the insecticides carbofuran, chlordane, malathion and methoxychlor; the herbicides 2,4-D, DEF, propanil and trifluralin; and the fungicide captan.

For each pesticide, the zoeal stages were found to be the most sensitive in long-term tests, approximately 5 to 10 times and 10 to 100 times more sensitive than juvenile and adult crabs, respectively, and were also affected at lower concentrations than those that affected egg hatching and prezoeal development. The maximum acceptable toxicant concentrations for continuous exposures of C. magister zoeae to each of the nine pesticides are: methoxychlor, 0.005 μg/liter; chlordane, 0.015 μg/liter; malathion, 0.02 μg/liter; carbofuran, 0.05 μg/liter; captan, 2 μg/liter; DEF, 4 μg/liter; trifluralin, 15 μg/liter; propanil, 80 μg/liter; and 2,4-D, 1000 μg/liter.

The toxicity of each of these pesticides to crabs is compared with literature reports of their toxicity to other aquatic species.

This report was submitted in fulfillment of Contract No. 68-01-0188 by Oregon State University under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period April 28, 1972 to April 28, 1974.

CONTENTS

Foreword iii
Abstract iv
Figures vi
Tables xii
Acknowledgments xiv

1. Introduction 1
2. Conclusions 2
3. Recommendations 3
4. Materials and Methods 4

Pesticides 4
Pesticide analytical methods 5
Experimental animals 5
Egg hatching - prezoeal bioassays 8
96-hr acute toxicity bioassays 9
Chronic toxicity bioassays 12
Uptake, loss and tissue distribution of methoxychlor. 21
Salinity tolerance experiment 21
Osmotic-ionic regulation experiments 22
ATPase assay 22

5. Results and Discussion 23

The fungicide captan 23
The insecticide methoxychlor 36
The herbicides 2,4-D, DEF, propanil and trifluralin . 62
The insecticides carbofuran, chlordane and malathion. 97
Summary of pesticide tolerance 112

References 119
Publications 125

FIGURES

Number | Page
1 Gas chromatogram of technical chlordane standard 6

2 Gas chromatogram of technical chlordane after extraction from seawater 7

3 Diagram of culture containers illustrating the relation between the square culture aquarium and the culture beakers, showing details of the automatic siphon, and the positioning of the two screens on the lower side of a culture beaker 14

4 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of captan concentration during a 24-hr exposure period 24

5 Effect of continuous exposure to captan on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973 27

6 Effect of continuous exposure to captan on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 28

7 Effect of continuous exposure to captan on molting of Dungeness crab zoeae beginning with the first zoeal molt 29

8 Effect of continuous exposure to captan on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs 30

9 Effect of continuous exposure to captan on molting of first instar Dungeness crabs to the second instar 31

Number Page

10 Effect of continuous exposure to captan on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs 32

11 Effect of continuous exposure to captan on survival of adult Dungeness crabs 33

12 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of methoxychlor concentration during a 24-hr exposure period 36

13 Effect of continuous exposure to methoxychlor on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973 38

14 Effect of continuous exposure to methoxychlor on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 39

15 Effect of continuous exposure to methoxychlor on molting of Dungeness crab zoeae beginning with the first zoeal molt 40

16 Effect of continuous exposure to methoxychlor on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs 41

17 Effect of continuous exposure to methoxychlor on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs 42

18 Effect of continuous exposure to methoxychlor on molting of first instar Dungeness crabs to the second instar 44

19 Effect of continuous exposure to methoxychlor on survival of adult Dungeness crabs 45

20 Whole body concentration of methoxychlor in juvenile crab, *Cancer magister*, as a function of time of exposure to the pesticide in seawater 50

21 Whole body concentration of methoxychlor in adult crabs, *Cancer magister*, as a function of time during and after a 15-day exposure to the pesticide in seawater 51

Number Page

22 Blood osmotic concentration of control and methoxychlor-treated Cancer magister as a function of medium osmolarity . . . 56

23 Blood and urine sodium concentration of control and methoxychlor-treated Cancer magister as a function of experimental salinity 57

24 Blood and urine potassium concentration of control and methoxychlor-treated Cancer magister as a function of experimental salinity 58

25 Blood and urine magnesium concentration of control and methoxychlor-treated Cancer magister as a function of experimental salinity 59

26 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of trifluralin concentration during a 24-hr exposure period 63

27 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of DEF concentration during a 24-hr exposure period 64

28 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of propanil concentration during a 24-hr exposure period 65

29 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of 2,4-D concentration during a 24-hr exposure period 66

30 Effect of continuous exposure to trifluralin on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973 68

31 Effect of continuous exposure to propanil on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973 69

32 Effect of continuous exposure to DEF on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973 70

Number Page

33 Effect of continuous exposure to 2,4-D on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973 71

34 Effect of continuous exposure to trifluralin on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 72

35 Effect of continuous exposure to propanil on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 73

36 Effect of continuous exposure to 2,4-D on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 74

37 Effect of continuous exposure to DEF on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 75

38 Effect of continuous exposure to trifluralin on molting of Dungeness crab zoeae beginning with the first zoeal molt 76

39 Effect of continuous exposure to DEF on molting of Dungeness crab zoeae beginning with the first zoeal molt 77

40 Effect of continuous exposure to propanil on molting of Dungeness crab zoeae beginning with the first zoeal molt 78

41 Effect of continuous exposure to 2,4-D on molting of Dungeness crab zoeae beginning with the first zoeal molt 79

42 Effect of continuous exposure to trifluralin on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs 80

43 Effect of continuous exposure to trifluralin on molting of first instar Dungeness crabs to the second instar 81

44 Effect of continuous exposure to trifluralin on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs 82

45 Effect of continuous exposure to DEF on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs 83

Number Page

46 Effect of continuous exposure to DEF on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs 84

47 Effect of continuous exposure to DEF on molting of first instar Dungeness crabs to the second instar 85

48 Effect of continuous exposure to propanil on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs 86

49 Effect of continuous exposure to propanil on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs 87

50 Effect of continuous exposure to propanil on molting of first instar Dungeness crabs to the second instar 88

51 Effect of continuous exposure to 2,4-D on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs 89

52 Effect of continuous exposure to 2,4-D on molting of first instar Dungeness crabs to the second instar 90

53 Effect of continuous exposure to 2,4-D on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs 91

54 Effect of continuous exposure to trifluralin on survival of adult Dungeness crabs 92

55 Effect of continuous exposure to 2,4-D on survival of adult Dungeness crabs 93

56 Effect of continuous exposure to DEF on survival of adult Dungeness crabs 94

57 Effect of continuous exposure to propanil on survival of adult Dungeness crabs 95

Number Page

58 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of carbofuran concentration during a 24-hr exposure period 97

59 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of chlordane concentration during a 24-hr exposure period 98

60 Percent hatch of Dungeness crab eggs, percent development of hatched crab larvae through the prezoeal to the first zoeal stage, and percent of developed first stage zoeae which are motile as a function of malathion concentration during a 24-hr exposure period 99

61 Effect of continuous exposure to carbofuran on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 101

62 Effect of continuous exposure to carbofuran on molting of Dungeness crab zoeae beginning with the first zoeal molt 102

63 Effect of continuous exposure to malathion on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 103

64 Effect of continuous exposure to malathion on molting of Dungeness crab zoeae beginning with the first zoeal molt 104

65 Effect of continuous exposure to chlordane on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974 105

66 Effect of continuous exposure to chlordane on molting of Dungeness crab zoeae beginning with the first zoeal molt 107

67 Effect of continuous exposure to carbofuran on survival of adult Dungeness crabs 108

68 Effect of continuous exposure to chlordane on survival of adult Dungeness crabs 109

69 Effect of continuous exposure to malathion on survival of adult Dungeness crabs 110

TABLES

Number | Page
1 Concentrations of Pesticides Used in Egg Hatching-Prezoeal Bioassay 10

2 Concentrations of Pesticides used in 96-hr Acute Toxicity Bioassays with First Instar Zoeae of *Cancer magister* 11

3 Concentrations of Pesticides used in 96-hr Acute Toxicity Bioassays with First Instar Juveniles of *Cancer magister* 12

4 Concentrations of Pesticides used in 96-hr Acute Toxicity Bioassays with Adults of *Cancer magister* 13

5 Pesticide Concentrations in Seawater during First Series of Zoeal Chronic Experiments 15

6 Pesticide Concentrations in Seawater during Second Series of Zoeal Chronic Experiments 16

7 Pesticide Concentrations in Seawater during First Series of Juvenile Chronic Experiments 18

8 Pesticide Concentrations in Seawater during Second Series of Juvenile Chronic Experiments 19

9 Pesticide Concentrations in Seawater during Adult Chronic Experiments 20

10 Decay Rate of Captan in Seawater of 25 °/₀₀ Salinity at 13°C 23

11 Acute Toxicity of Captan to First Instar Zoeae, First Instar Juvenile Crabs and Adult Crabs 26

12 Acute Toxicity of Methoxychlor to First Instar Zoeae, First Instar Juvenile Crabs and Adult Crabs 37

13 The Effect of Methoxychlor on Survival of Molted Juvenile and Adult Crabs during Continuous Exposure to the Pesticide for 80 and 85 days, Respectively 43

Number		Page
14	Whole Body Methoxychlor Concentrations in Adult Crabs Killed during Continuous Exposure to Methoxychlor for up to 15 days	48
15	Whole Body Residues of Methoxychlor in Adult Crabs washed with Water Only or with Water First, followed by Acetone before Pesticide Analysis	49
16	Distribution of Methoxychlor in Selected Tissue of Adult Crabs after 15 Days of Continuous Exposure to 1.8 or 7.5 µg/liter of the Pesticide in Seawater	52
17	Percent Mortality of Adult Crabs Exposed to Methoxychlor in Dilute Seawater	54
18	ATPase Activity in Gills of Adult Crabs exposed to Methoxychlor	61
19	Acute Toxicity of Herbicides to First Instar Zoeae, First Instar Juvenile Crabs and Adult Crabs in 96-hr Tests	67
20	Acute Toxicity of Insecticides to First Instar Zoeae and Adult Crabs in 96-hr Tests	100
21	Toxic Concentrations of Various Insecticides to Larval Crabs	113
22	Relative Sensitivity of Various Life History Stages of _C. magister_ to Nine Pesticides	114
23	Estimated Maximum Acceptable Toxicant Concentrations for Continuous Exposure of Crabs to Each of Nine Pesticides	118

ACKNOWLEDGMENTS

The author is especially grateful to Mr. David V. Buchanan, Mr. David A. Armstrong and Mr. Michael H. Mallon for substantial contributions in the design and conduct of various parts of the project studies and also for contributions to the writing of this report. Able technical assistance was also provided by Mr. Michael J. Myers and Ms. Barbara E. Stone. Professor Wilbur P. Breese, Mr. Nelson E. Stewart and Dr. Peter Doudoroff often contributed sound advice.

For contributions of pesticide chemicals used in the studies we thank Eli Lilly and Company; Rohm and Haas Company; the Chemagro Division of Baychem Corporation; Miller's Products of Portland, Oregon; FMC Corporation; Chevron Chemical Company; and E. I. DuPont De Nemours and Company.

SECTION I

INTRODUCTION

The widespread use of pesticides in recent years has led to concern over the consequences of such usage for aquatic organisms. Until recently, the majority of aquatic toxicology studies have dealt with freshwater species, have emphasized the accumulation of acute toxicity data and have frequently been limited to studies with adult organisms. In the present study, we have examined the acute and chronic toxicity of nine pesticides to various developmental stages of the Dungeness crab, *Cancer magister* Dana, an important commercial species of the west coast of North America. The compounds tested were the fungicide captan; the herbicides 2,4-D, DEF, propanil and trifluralin; and the insecticides carbofuran, chlordane, malathion and methoxychlor.

Our objective was to establish for each pesticide the highest concentration having no discernible effect on *C. magister* during prolonged exposures on the assumption that these concentrations could be applied as criteria for protection of the species in marine waters. Mount and Stephen (1967) have recommended procedures for establishing the maximum acceptable toxicant concentration for aquatic species. These involve exposure of organisms to constant concentrations of a toxicant throughout an entire life cycle, thus providing an opportunity for assessment of effects on all stages of the life history including the potentially sensitive reproductive processes. Comparable studies with *C. magister* were not feasible since this species requires from 3 to 4 years for completion of its life cycle (Butler, 1961). Our approach, therefore, was to expose zoeal, juvenile and adult stages of the crab, in separate experiments lasting up to 80 days, to uniform concentrations of each pesticide in water and determine for each developmental stage the maximum tolerable concentration of each compound. Tolerable concentrations were those which had no effect on survival and also, in the case of zoeae, had no effect on the timing of the zoeal molts. In studies with methoxychlor, additional sublethal criteria having to do with water and salt balance in adults were also employed. Studies involving short-term exposures to each pesticide were also used to examine effects on the hatching of eggs and the metamorphosis of prezoeae to the first zoeal stage.

SECTION 2

CONCLUSIONS

Tolerance of zoeal, juvenile and adult stages of the Dungeness crab, Cancer magister, to each pesticide was lower in long-term tests than in acute tests. The ratios of "no effect" concentrations for zoeae in chronic tests to the 96-hr LC50s for zoeae ranged from <0.0002 for captan to 0.61 for DEF.

The earliest developmental sequence of C. magister examined in this study, encompassing the period from just before egg hatching to just after development into the first zoeal instar, is not affected by pesticide concentrations having an adverse effect on zoeae in long-term exposures. Therefore, effects on this sequence of development need only be considered when short-term pesticide exposures are anticipated.

In long-term tests, the zoeal stages of C. magister were approximately 5 to 10 times more sensitive to the pesticides studied than juvenile stages and were approximately 10 to 100 times more sensitive than adult stages. Zoeae should be used, therefore, in deriving water quality criteria for this species.

Sublethal effects of pesticide exposures were either not observed, or were noted only at concentrations or exposure times slightly less than those that affected survival for any stage. Additional sublethal effects studies do not, therefore, appear to be necessary for the establishment of criteria for the protection of C. magister exposed to the nine pesticides examined in this study.

The lowest toxic concentrations of methoxychlor, chlordane and carbofuran in long-term tests with larval C. magister are comparable to those reported previously for related insecticides in long-term tests with crab zoeae. Data are lacking with which to make similar comparisons for the insecticide malathion, the fungicide captan, and the herbicides 2,4-D, DEF, propanil and trifluralin.

Our studies indicate that the maximum acceptable toxicant concentrations for continuous exposures of C. magister to each of the nine pesticides are: methoxychlor, 0.005 μg/liter; chlordane, 0.015 μg/liter; malathion, 0.02 μg/liter; carbofuran, 0.05 μg/liter; captan, 2 μg/liter; DEF, 4 μg/liter; trifluralin, 15 μg/liter; propanil, 80 μg/liter; and 2,4-D acid, 1000 μg/liter. As defined in this study, the maximum acceptable toxicant concentration is the highest concentration of each pesticide tested individually for which no lethal or sublethal effects were observed during either acute or chronic exposures of zoeae, juveniles or adults, or during acute exposures of eggs and prezoeae.

SECTION 3

RECOMMENDATIONS

Additional studies to examine the effects of these nine pesticides on reproduction in *Cancer magister* would be desirable to ensure that this process is not more sensitive to pesticides than zoeal development. Such studies should include exposure of male and female crabs to pesticides prior to mating and then subsequent exposure of the females and their progeny well into the zoeal stages. Criteria of effects for these studies should include viability of the eggs and resulting zoeae and should be evaluated in terms of the exposure concentrations and residue levels in crabs, eggs and resulting larvae.

The toxicity experiments with chlordane suggest that this compound may be biologically stable and progressively accumulated in crabs. Because of the high toxicity of chlordane, these properties should be further examined.

Initial evaluations of the effects in crabs of pesticides other than those examined here should be limited to studies on the survival and molt inhibition of zoeal stages since, in the absence of further knowledge of reproductive effects, these experiments seem to provide the most sensitive assessment of effects.

Based on our results and on our review of the literature for the nine pesticides examined in this study, we believe that the tolerance of larval crabs to other pesticides would often be as low or lower than that of other marine or freshwater organisms. We recommend, therefore, that consideration be given to the use of larval crab bioassays as a preferred means of establishing water quality criteria for the protection of other marine species.

We recommend that the concentrations of the nine pesticides in marine waters not be allowed to exceed the maximum acceptable toxicant concentrations established in this study (Table 23).

SECTION 4

MATERIALS AND METHODS

PESTICIDES

The list of pesticides studied, their sources and the formulations tested were the following:

1. Trifluralin; α,α,α-Trifluoro-1,6-dinitro-N,N-dipropyl-p-toluidine; Eli Lilly and Company; Technical, Lot No. 5GB70, purity 93%; Treflan® E.C., Lot No. 8759B, active ingredient 44.5%.
2. DEF®; S,S,S,-Tributyl phosphorotrithioate; Chemagro Corporation; Technical, Lot No. 3050106, purity 94%.
3. Propanil; 3,4-Dichloropropionanilide; Rohm and Haas; Technical, Lot No. 2-8262, purity 82%; Stam F-34, Lots No. 2-8469 and 2-3078, active ingredient 35%.
4. 2,4-D; 2,4-Dichlorophenoxyacetic acid; Aldrich Chemical Company; Technical, Lot No. 070117, purity 98%.
5. Captan; N-(trichloromethylmercapto)-4-cyclohexene-1,2-dicarboximide; Chevron Chemical Company; Technical, Lot No. not specified, purity 92.8%; Orthocide-50W, Lot No. PN1760, active ingredient 50%.
6. Methoxychlor; 2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane; E. I. DuPont De Nemours and Company; Technical, Lot No. not specified, purity 92%.
7. Carbofuran; 2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate; FMC Corporation; Technical, Lot No. ME L514 (C4717-54-A).
8. Malathion; O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate; American Cyanamid Company; Technical (Cythion grade), Lot No. not specified, purity 95.0%.
9. Chlordane; 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene; Lot No. not specified.

PESTICIDE ANALYTICAL METHODS

Gas chromatographic procedures were employed for the analysis of each of the pesticides in this study. The instrument used was a Hewlett-Packard Model 5713A gas chromatograph equipped with a ^{63}Ni electron capture detector and a glass column 1.83 m x 4 mm. The column packing was 3.8% UCW-98 on 80/100 mesh Chromosorb W-HP. The carrier gas was 5% methane in argon used at a flow rate of 60 ml/min. Oven and detector temperatures generally were 220°C and 300°C, respectively. The injection volume was 5 µl.

Methoxychlor, chlordane, malathion, propanil, DEF and trifluralin in seawater were analyzed directly following solvent extraction into hexane. The solvent extractions of captan and carbofuran employed benzene rather than hexane. The 2,4-dinitrophenyl ether derivative of carbofuran was prepared by a modification of the method of Holden (1973) prior to chromatographic analysis. A satisfactory analytical procedure for 2,4-D in seawater was not developed and consequently no analyses for this compound were attempted.

Quantitation in the case of each pesticide was based upon standards obtained from EPA (Quality Assurance Section, EPA, Pesticides & Toxic Substances Effects Lab., Chemstrand Building, Research Triangle Park, N.C. 27711; formerly the Perrine Lab.), with the exception of trifluralin, which was obtained from the manufacturer (Eli Lilly & Co.), and chlordane. The latter, which is a mixture of α-chlordane, γ-chlordane, heptachlor and other related compounds, was quantitated by comparing the height of the α-chlordane peak with that of a weighed sample of the technical material dissolved in hexane. There was no evidence that chlordane extracted from water differed in composition from the technical mixture; chromatograms of the water extracts were similar to those of the technical chlordane used as a standard (Figures 1 and 2).

Methoxychlor residues in whole crab and excised tissues were also determined by gas chromatography following solvent extraction of the homogenized tissues. The procedure involved a 15-min extraction of 2 g of homogenized tissue with 10 ml of glass redistilled acetone followed by an additional 15-min extraction with shaking after the addition of 20 ml of glass redistilled hexane. The extract was then shaken with 20 ml of hexane-extracted glass-distilled water. From the hexane layer, 2 ml were taken for further cleanup on a florisil column (3 g activated florisil contained in a 10 mm I.D. glass chromatography tube). Methoxychlor was eluted from the column with 40 ml of 5% ether in hexane and the eluate was chromatographed without further treatment. A five-fold increase in florisil weight and eluant volume were needed to effect an adequate cleanup of the fatty hepatopancreas and gonad tissues. Recoveries estimated by the method of addition averaged 83% and ranged from 70 to 100%. The data as presented in this report do not include a recovery correction.

EXPERIMENTAL ANIMALS

Toxicity experiments were conducted utilizing four developmental stages in the life history of the Dungeness crab (*Cancer magister* Dana). These were

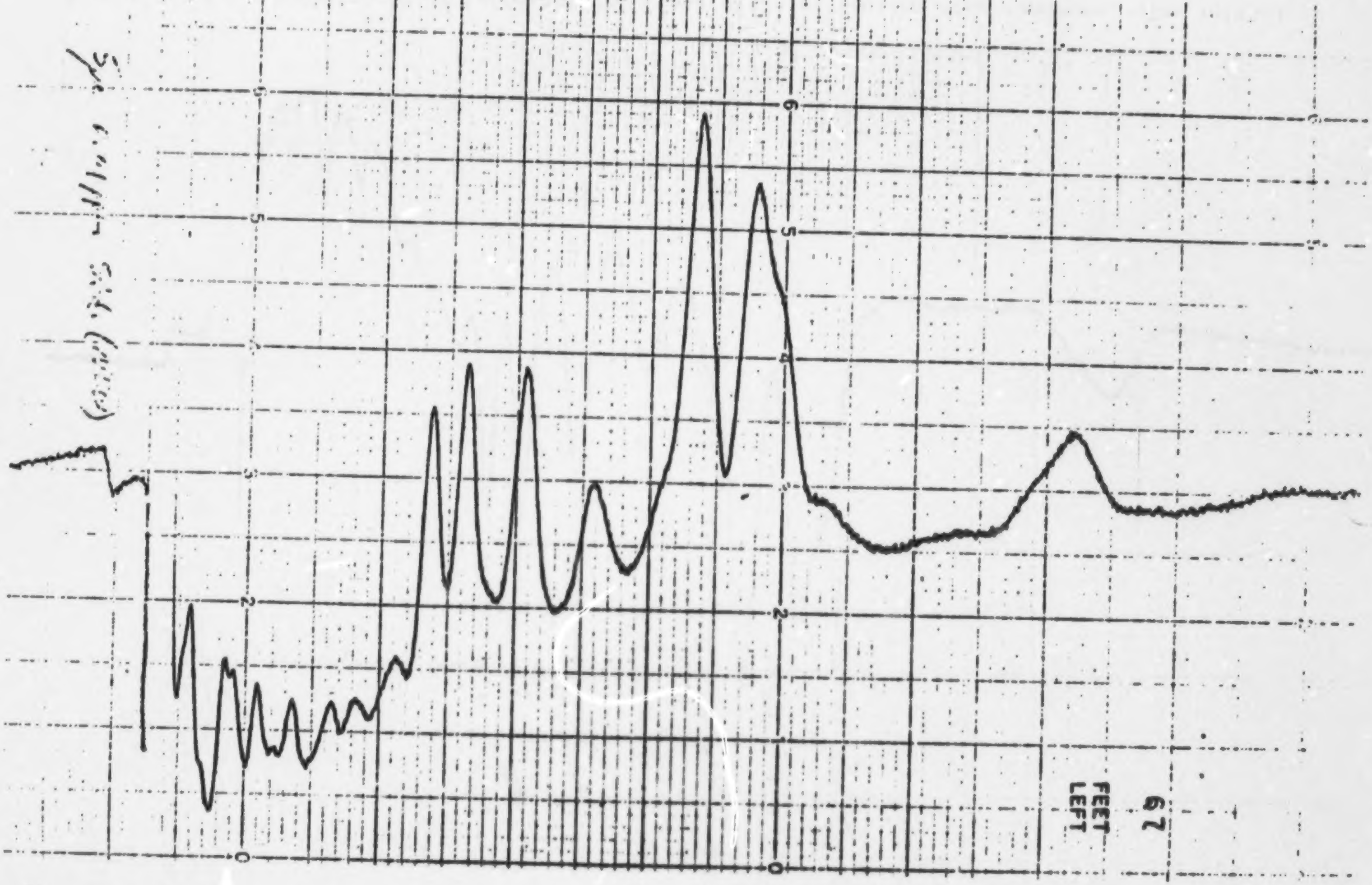


Figure 1. Gas chromatogram of technical chlordane standard.

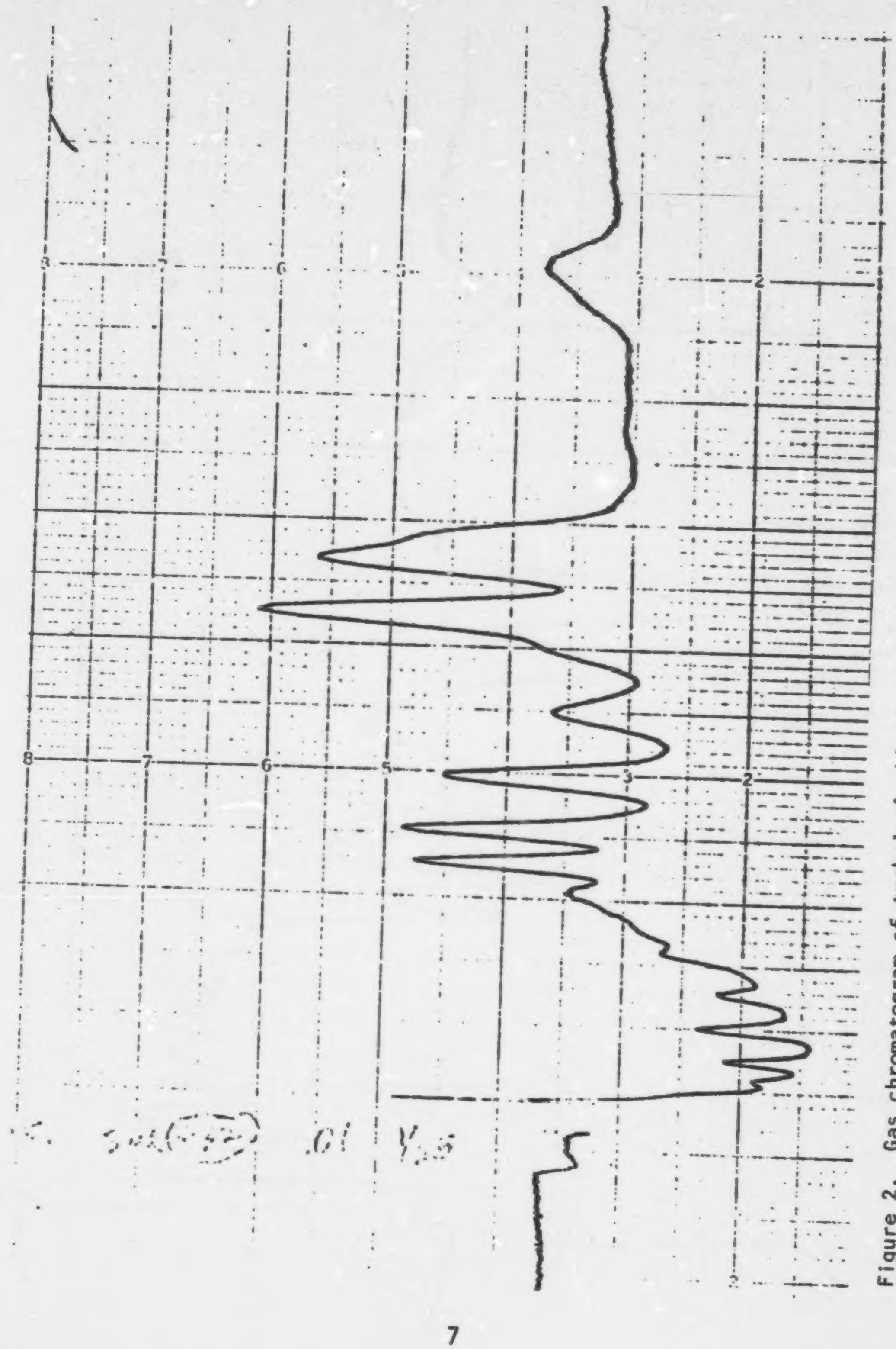


Figure 2. Gas chromatogram of technical chlordane after extraction from seawater. The initial concentration of the technical material in seawater was 10 µg/liter.

the egg hatching - prezoeal stage, the zoeal stage, the early postlarval or juvenile crab stage and the adult stage.

Ovigerous female crabs were collected as the source of eggs and zoeae for tests on the first two developmental stages. The ovigerous female crabs were collected in the ocean off Newport by commercial fishermen during winter and early spring and were returned live to the laboratory where they were maintained in tanks of flowing seawater for variable periods of time depending on the stage of ova development. The crabs, when maintained in the laboratory in excess of a week, were fed frozen fillets of starry flounder (Platichthys stellatus). To obtain the eggs for use in the egg hatching bioassay, unhatched eggs attached to the egg mass of females, in which initial hatching had been observed, were randomly selected and gently removed from the crab and placed directly in the bioassay containers. The procedure for obtaining first stage zoeae for use in the zoeal bioassays was slightly different. For these experiments, at the onset of hatching the female was transferred to standing, ultraviolet light-sterilized seawater at 13°C and 25 °/₀₀ or 30 °/₀₀ salinity. Within 24 hr, swimming first-instar zoeae were collected in beakers and used immediately in the bioassay tests.

Large numbers of juvenile crabs at the same developmental stages were conveniently obtained by collecting megalopae swarming in the bays or nearshore ocean areas. The megalopae were held in tanks of running seawater in the laboratory where most metamorphosed into first-instar crabs within 3 to 4 days. In the 96-hr bioassay tests and the first chronic toxicity bioassay series, juvenile crabs were used within 4 days of metamorphosis. However, in the second chronic bioassay series the crabs were held in the laboratory for approximately 2 months in natural flowing seawater prior to use. These crabs were maintained in a tray with a sand substratum and were fed cockle clams and rockfish at least weekly.

Adult crabs were collected by trawl from Yaquina Bay, Oregon. These crabs, which ranged in size from 80 to 100 mm in carapace width, were probably 10th or 11th instars, about 2 years old, and approaching sexual maturity (Butler, 1961). Slightly larger crabs, averaging 165 g and 107 mm in carapace width, were used in the experiments on methoxychlor uptake.

EGG HATCHING - PREZOEAL BIOASSAYS

Buchanan and Millemann (1969) have described a free prezoeal stage of 5 to 15 min duration in the normal development of C. magister. The possibility exists that this stage or the egg hatching process itself may have a greater susceptability to certain of the pesticides than would the later developmental stages. A series of experiments were conducted to evaluate this possibility. Because of the short duration of the prezoeal stage, a 24-hr exposure period was considered adequate.

An ovigerous crab was caught in the ocean off Newport on March 10, 1974, and held for 2 days in the laboratory in a 31-gal tank supplied with flowing seawater at 11° to 12°C and 25 to 30 °/₀₀ salinity. At the time of initial hatching, unhatched eggs, with all their cuticular layers intact, were randomly selected and then gently removed from the crab and placed approximately 30 to a beaker in 250 ml beakers containing 200 ml of test solution. A logarithmic series of concentrations, spanning at least two orders of magnitude, were tested for each of the pesticide compounds (Table 1). Each pesticide concentration was tested in duplicate beakers giving a total of approximately 60 eggs per test concentration. The pesticides were added to seawater by first dissolving in acetone and then stirring in an appropriate volume of the acetone stock solution to give a final acetone concentration of 100 µl/liter. Control incubations were tested with and without acetone. Each control series consisted of four separate beakers and a total of about 120 eggs. The numbers of unhatched eggs, prezoeae, and first instar zoeae in the test vessels, and the percentage of first stage zoeae which were motile, were recorded at the end of the 24-hr exposure period. During these exposures, the dissolved oxygen remained at air saturation, the salinity was 30 °/₀₀ and the temperature was 12° to 13°C.

96-HR ACUTE TOXICITY BIOASSAYS

Acute toxicity bioassays lasting up to 96 hr were conducted with each of the pesticides using first instar zoeae, first instar juvenile crabs and adult crabs. The acute toxicity experiments were analyzed according to the straight line graphical interpolation method (American Public Health Association et al.,1971). The results are expressed either as the EC50, the concentration of pesticide that produced a nonlethal response in 50% of the test organisms in 24, 48 or 96 hr, or the LC50, the concentration of pesticide that was lethal to 50% of the test organisms during the same time periods. The criterion of death for juvenile and adult crabs was absence of movement after stimulation, and for zoeae, opaqueness, an unmistakable indication of death obvious within 1 to 6 hr. The nonlethal response used for juveniles was inability to right from an overturned position; for zoeae, it was inhibition of swimming. A nonlethal response was not employed in the tests with adult crabs.

In each of two separate series of acute toxicity bioassays with zoeae, in which different groups of pesticides were examined, the larvae were pooled from two female crabs that had been held in the laboratory in flowing seawater for from 5 to 12 days. First instar juvenile crabs were obtained from wild megalopae which were allowed to metamorphose in the laboratory in flowing seawater tanks at 13° ± 2°C. The time from collection of magalopae to use of juvenile crabs in the acute toxicity bioassays was 4 days. After collection, adult crabs were acclimated for 5 days to 13° ± 1°C and 25 ± 0.5 °/₀₀ salinity before initiating the acute toxicity bioassays. None of the stages of crabs were fed in the laboratory prior to initiating the tests.

All acute toxicity bioassays utilized static water conditions in glass containers; 250 ml beakers holding 200 ml of test solution for zoeae and juveniles, and 12-liter glass jars holding 10 liters of test solution for adults. All bioassays were conducted at 13° ± 1°C and 25 ± 0.5 °/₀₀ salinity. The formulations and concentrations of pesticides tested are given in Tables 2, 3 & 4. The pesticides were dissolved in acetone to facilitate

TABLE 1. CONCENTRATIONS OF PESTICIDES USED IN EGG HATCHING - PREZOEAL BIOASSAYS

Pesticide	Test concentration (µg/liter) *					
	1	2	3	4	5	6
Carbofuran	0.33	0.10	0.033	0.010	0.0033	0.0010
Chlordane	0.33	0.10	0.033	0.010	0.0033	0.0010
Malathion	0.10	0.033	0.010	0.0033	0.0010	0.00033
Methoxychlor	0.01	0.0033	0.0010	0.00033	0.00010	0.000033
Captan	10.	3.3	1.0	0.33	0.10	-
DEF	0.10	0.033	0.010	0.0033	0.0010	0.00033
Propanil (Stam F-34)	33.	10.	3.3	1.0	0.33	0.10
2,4-D acid	330.	100.	33.	10.	3.3	-
Trifluralin	0.33	0.10	0.033	0.010	0.0033	-

* Nominal concentrations obtained by dilution of stock solutions.

TABLE 2. CONCENTRATIONS OF PESTICIDES USED IN 96-HR ACUTE TOXICITY BIOASSAYS WITH FIRST INSTAR ZOEAE OF *CANCER MAGISTER*

Pesticide	Test concentration (μg/liter) *							
	1	2	3	4	5	6	7	8
Technical Grade								
Carbofuran	10	3.3	1.0	0.33	0.10	0.033	-	-
Chlordane	10	3.3	1.0	0.33	0.10	0.033	-	-
Malathion	10	3.3	1.0	0.33	0.10	0.033	-	-
Methoxychlor	0.90	0.29	0.090	0.029	0.0090	0.0029	0.00090	-
Captan	10,000	3,300	1,000	330	100	33	-	-
DEF	1,000	330	100	33	10	3.3	1.0	0.33
Propanil	15,000	4,800	1,500	480	150	48	-	-
2,4-D acid	10,000	3,300	1,000	330	100	33	-	-
Trifluralin	110	35	11	3.5	1.1	0.35	-	-
Formulations								
Orthocide - 50W	10,000	3,300	1,000	330	100	33	-	-
Stam F-34	100,000	33,000	10,000	3,300	1,000	330	-	-
Treflan E.C.	330	100	33	10	3.3	1.0	-	-

* Nominal concentrations obtained by dilution of stock solutions.

TABLE 3. CONCENTRATIONS OF PESTICIDES USED IN 96-HR ACUTE TOXICITY BIOASSAYS WITH FIRST INSTAR JUVENILES OF CANCER MAGISTER

Pesticide	Test concentration (µg/liter) *					
	1	2	3	4	5	6
Methoxychlor	100	33	10	3.3	1.0	0.33
Captan	10,000	3,300	1,000	330	100	33
DEF	1,000	330	100	33	10	3.3
Propanil (Stam F-34)	33,000	10,000	3,300	1,000	330	100
2,4-D acid	100,000	33,000	10,000	3,300	1,000	330
Trifluralin	1,000	330	100	33	10	3.3

* Nominal concentrations obtained by dilution of stock solutions.

mixing with seawater. The final acetone concentration was 100 µl/liter except for the captan tests with adult crabs in which the final acetone concentration was 1,000 µl/liter. Control animals were held in untreated seawater and in seawater containing acetone. The numbers of animals per test concentration were 20, 10 and 10 for zoeae, juveniles and adults, respectively. The test solutions were renewed daily after first determining the dissolved oxygen levels and pH of the 24-hr-old water. Solutions were aerated only in the adult tests. Dissolved oxygen levels always exceeded 7.0 and 5.5 mg/liter in the zoeal and juvenile tests, respectively, and averaged 6.0 mg/liter in tests with adults. The averages of pH for the same three groups was 7.8, 7.8 and 7.5. The photoperiod approximated that occurring naturally during the time the tests were conducted. These were 9 hr of light - 15 hr of darkness for the zoeal tests, 15 hr of light - 9 hr of darkness for the juvenile tests, and 12 hr of light - 12 hr of darkness for the adult tests.

CHRONIC TOXICITY BIOASSAYS

Long-term exposures of zoeae, juvenile crabs and adult crabs to pesticides were conducted in flowing-water culture systems. Pesticides were introduced into the culture water by metering acetone stock solutions of the chemicals into the upper ends of cascading mixing troughs. The final seawater concentration of acetone in all tests was 100 µl/liter. Brinkmann multi-channel peristaltic metering pumps were used to deliver the acetone stock solutions. The flow of seawater into the mixing troughs was regulated by using adjustable hose clamps and constant level headtanks. Careful monitoring and periodic adjustment of seawater flow rates and acetone metering rates was necessary to provide uniform pesticide concentrations in seawater. Failsafe devices were employed to ensure that the metering of pesticides were halted if the supply of seawater failed. All seawater was filtered through large quartz sand filter beds before use in the bioassays. Additional filtration and UV sterilization were employed in all of the juvenile crab and

TABLE 4. CONCENTRATIONS OF PESTICIDES USED IN 96-HR ACUTE TOXICITY BIOASSAYS WITH ADULTS OF CANCER MAGISTER

Pesticide	Test concentration (μg/liter) *					
	1	2	3	4	5	6
Carbofuran	1,000	330	100	33	10	-
Chlordane	3,300	1,000	330	100	33	-
Malathion	3,300	1,000	330	100	33	-
Methoxychlor	920	520	290	160	92	29
Captan	100,000	33,000	10,000	3,300	1,000	330
DEF	1,000	330	100	33	10	3.3
Propanil	26,000	8,200	2,600	820	260	82
2,4-D acid	-	-	-	-	-	-
Trifluralin	9,300	3,000	930	300	93	30

* Nominal concentrations obtained by dilution of stock solutions.

zoeal tests. During the 1974 zoeal tests, a third filter, capable of removing 10 μm particles, and a second UV sterilization treatment step were also employed. The latter precautions were taken to further reduce the possibility of infections of zoeae by microorganisms.

Zoeal Experiments

Although the basic bioassay technique used in all of the chronic toxicity experiments was essentially the same, the practical problems of culturing the organisms differed for the various developmental stages and required separate solutions. In previous long-term toxicity studies with brachyuran larvae (Bookhout et al., 1972; Buchanan et al., 1970; Epifanio, 1971) investigators have employed static culture vessels which required periodic renewal of the test solutions by manual procedures. In order to utilize a flowing-water system, we developed the culture apparatus illustrated in Figure 3. Crab larvae were contained in each of several 250 ml glass beakers modified with a 15 mm diameter hole on the side near the bottom of the beaker. Nylon screen, 360 μm mesh "Nitex," was cemented, by using silicone rubber sealant, across the opening on the inside of the beaker. A second screen, 210 μm mesh "Nitex," was cemented in the same manner across the opening on the outside of the beaker so that the two screens were separated by a distance of about 3 to 4 mm. As many as eight culture beakers could be contained in each 28 cm by 28 cm by 10 cm deep glass aquarium. Seawater containing toxicant of the desired concentration entered each aquarium through a 4 mm inside diameter glass tube. By means of an automatic siphon, the water level in the aquarium was made to fluctuate, causing seawater to enter and exit the beakers through the screened holes.

Two series of chronic toxicity experiments were conducted with zoeae. The first utilized the progeny of a single female collected on April 12, 1973. At the onset of hatching 1 day later, the female was placed in

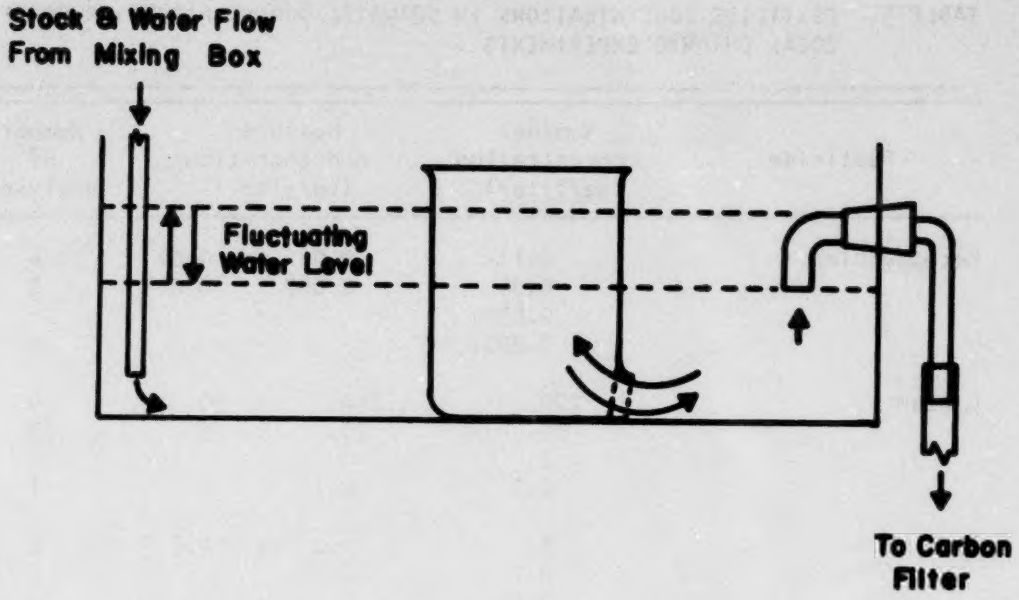


Figure 3. Diagram of culture containers illustrating the relation between the square culture aquarium and the culture beakers, showing details of the automatic siphon, and the positioning of the two screens on the lower side of a culture beaker.

standing UV treated seawater at 13° ± 1°C and 25 °/₀₀ salinity. First instar zoeae were collected 5 to 10 hr after hatching and placed 10 each in the 250-ml culture beakers. The pesticides used and their concentrations are given in Table 5. A total of 80 larvae were tested with each pesticide concentration. Twice per week, following transfer to clean culture beakers, zoeae were fed 700 to 1,000 newly hatched San Francisco brine shrimp (Artemia salina) nauplii per beaker. The seawater salinity during the first 15 days of the test was 25 °/₀₀ and afterwards was 15 °/₀₀ or higher. Temperature was maintained at 13° ± 1°C. The dissolved oxygen levels always exceeded 8.0 mg/liter and the pH ranged from 7.6 to 7.9. The photoperiod was 12.5 hr of light - 10.5 hr of darkness.

The second series of chronic toxicity tests was also conducted with the progeny of a single female which was obtained from a commercial fisherman on March 10, 1974. The ovigerous female was held in the laboratory in flowing seawater at 11° to 12°C for 2 days. At the onset of hatching the female was transferred to standing UV sterilized seawater at 30 °/₀₀ salinity and, after 10 hr, swimming first stage zoeae were collected for use in the bioassays. For each pesticide concentration used (Table 6), 10 zoeae were placed in each of four 250 ml beakers giving a total of 40 larvae per pesticide concentration. Zoeae were fed first naupliar stages of Artemia salina three times

TABLE 5. PESTICIDE CONCENTRATIONS IN SEAWATER DURING FIRST SERIES OF ZOEAL CHRONIC EXPERIMENTS

Pesticide	Nominal concentration (µg/liter)	Measured concentration (µg/liter)	Number of analyses
Methoxychlor	0.1	0.06 ± 0.02*	4
	0.01	0.007 ± 0.004	3
	0.001	-	-
	0.0001	-	-
Captan	200.	240. ± 90.	4
	20.	20. ± 5.	3
	2.	-	-
	0.2	0.18	1
DEF	2.	1.2 ± 0.3	2
	0.2	0.70	1
	0.02	-	-
	0.002	-	-
Propanil (Stam F-34)	800.	1,400. ± 200.	3
	80.	150. ± 0.	2
	8.	<2.	3
	0.8	-	-
2,4-D acid	10,000.	-	-
	1,000.	-	-
	100.	-	-
	10.	-	-
Trifluralin	150.	480. ± 40.	2
	15.	53. ± 15.	3
	1.5	4.1 ± 0.6	4
	0.15	-	-

* Mean ± one standard deviation.

per week at a feeding density of 700 brine shrimp per beaker for the first to third instar zoeae, and 1,000 brine shrimp per beaker for later zoeal stages. Zoeae were transferred to clean beakers three times a week. The photoperiod was adjusted monthly to approximate ambient conditions and was 11 hr of light - 13 hr of darkness, 13.5 hr of light - 10.5 hr of darkness, and 15 hr of light - 9 hr of darkness for the months of March, April and May, respectively. The mean and standard deviation of culture temperatures recorded daily was 12.3° and 0.5°C. The salinity recorded daily was 28.8 ± 1.4 ‰ and the dissolved oxygen and pH recorded weekly were 8.7 ± 0.3 mg/liter and 8.1 ± 0.1, respectively. Mortality and molt data were recorded daily.

TABLE 6. PESTICIDE CONCENTRATIONS IN SEAWATER DURING SECOND SERIES OF ZOEAL CHRONIC EXPERIMENTS

Pesticide	Nominal concentration (µg/liter)	Measured concentration (µg/liter)	Number of analyses
Carbofuran	5.	7.3 ± 1.3*	2
	0.5	0.63 ± 0.32	8
	0.05	-	-
Chlordane	15.	62.	1
	1.5	1.70 ± 0.28	2
	0.15	0.17 ± 0.09	11
	0.015	-	-
Malathion	2.	-	-
	0.2	-	-
	0.02	-	-
	0.002	-	-
Methoxychlor	0.5	0.91 ± 0.39	6
	0.05	0.074 ± 0.019	13
	0.005	-	-
Captan	200.	450. ± 80.	6
	20.	30. ± 11.	8
	2.	3.1 ± 2.7	14
DEF	4.	6.9 ± 2.4	13
	0.4	0.95 ± 0.24	13
	0.04	-	-
Propanil (Stam F-34)	800.	1,700. ± 500.	12
	80.	-	-
	8.	9.6 ± 3.6	14
2,4-D acid	10,000.	-	-
	1,000.	-	-
Trifluralin	150.	220. ± 50.	2
	15.	26. ± 5.	11
	1.5	3.1 ± 0.3	14

* Mean ± one standard deviation.

Juvenile Experiments

Two series of chronic toxicity bioassays were done with juvenile crabs, the first lasting 36 days and the second 80 days. Wild megalopae collected in Yaquina Bay, Oregon between June 12 and June 15, 1973 were held in the laboratory in flowing seawater at 15° ± 2°C and at variable salinities exceeding 25 °/₀₀ until they had molted into first instar crabs. Some of the crabs were used immediately in the first series of experiments. The remaining crabs, which were used in the second series of tests, were held in flowing seawater in the laboratory for approximately 2 months and fed cockle clams (Clinocardium nuttalli) and rockfish (Sebastodes sp.) at least once a week.

In the first experiments, crabs were reared in the same apparatus used in the long-term tests with zoeae except that each beaker was divided by a glass partition into two separate chambers. Each chamber contained one crab which was thus protected from cannibalism. As the first crabs began to molt in this experiment, we noticed that the animals were experiencing difficulty in completing the molt. In earlier experiments, Buchanan et al. (1970) found that crabs of this age were able to molt with good survival when a sandy substratum was present. On day 15, therefore, all the crabs were removed from the glass beakers and allowed to intermix in the rearing troughs, to which was added clean beach sand to a depth of 1.0 cm.

Four concentrations of each pesticide were used in the first series of experiments (Table 7). Control animals received either untreated seawater or seawater containing 100 μl/liter of acetone. During the experiment, seawater temperatures recorded daily were 13° ± 2°C, and the salinity was 31 to 34 °/₀₀. Daily determinations of dissolved oxygen and pH gave values ranging from 8.0 to 8.7 mg/liter and from 7.3 to 8.1, respectively. The photoperiod was 14 hr of light - 10 hr of darkness.

Juvenile crabs used in the second series of tests were mostly in the third crab instar by the time the tests were initiated. Fewer concentrations of some of the pesticides were used (Table 8), and acetone and seawater controls were employed as before. In these experiments, the crabs were reared directly in the large exposure troughs that contained 2 cm of beach sand as a substratum. By using Teflon-coated screens, each aquarium was, in these experiments, divided into 30 cm^2 compartments each containing one crab. The sand was replaced and the screens cleaned on test days 28 and 58. Dissolved oxygen levels monitored daily ranged from 8.0 to 8.7 mg/liter, and the daily pH reading ranged from 7.3 to 8.1. Seawater temperatures and salinities recorded daily were 13° ± 1°C and 32 to 34.5 °/₀₀, respectively. The photoperiod used was the same as in the first series of juvenile tests. Crabs in both the first and second series of tests were fed to repletion small pieces of cockle clam on alternate days and daily observations were made on their survival and molting.

Adult Experiments

In an initial series of adult chronic toxicity experiments, lasting 85 days, crabs were exposed to from two to four concentrations of the pesticides

TABLE 7. PESTICIDE CONCENTRATIONS IN SEAWATER DURING FIRST SERIES OF JUVENILE CHRONIC EXPERIMENTS

Pesticide	Nominal concentration (μg/liter)	Measured concentration (μg/liter)	Number of analyses
Methoxychlor	4.	4.9 ± 1.3*	3
	0.4	0.45 ± 0.24	3
	0.04	0.033 ± 0.017	3
	0.004	-	-
Captan	200.	510. ± 160.	3
	20.	51. ± 14.	2
	2.	1.5 ± 0.4	2
	0.2	0.20 ± 0.14	2
DEF	150.	260. ± 70.	3
	15.	25. ± 9.	3
	1.5	2.7 ± 0.6	3
	0.15	-	-
Propanil (Stam F-34)	800.	1,200. ± 200.	3
	80.	130. ± 80.	3
	8.	8	1
	0.8	-	-
2,4-D acid	10,000.	-	-
	1,000.	-	-
	100.	-	-
	10.	-	-
Trifluralin	150.	190. ± 40.	2
	15.	19. ± 2.	2
	1.5	2.6 ± 0.7	3
	0.15	-	-

* Mean ± one standard deviation.

methoxychlor, 2,4-D, trifluralin, DEF, propanil (as Stam F-34) and captan (Table 9). Ten crabs, five males and five females, were exposed to each pesticide concentration. Each of the two control groups, one exposed to uncontaminated seawater and the other to seawater containing 100 μl/liter of acetone, consisted of 30 crabs, half male and half female. The crabs were held in 47-liter aquaria which were partitioned sequentially into five interconnected compartments. The dimensions of each compartment were 30 x 21 x 15 cm, and each contained one crab. Seawater entered each aquarium at a rate of 1.6 to 2.0 liters/min giving a weight specific replacement rate of about 2.6 liters/g/day.

TABLE 8. PESTICIDE CONCENTRATIONS IN SEAWATER DURING SECOND SERIES OF JUVENILE CHRONIC EXPERIMENTS

Pesticide	Nominal concentration (μg/liter)	Measured concentration (μg/liter)	Number of analyses
Methoxychlor	4.	6.5 ± 4.7*	6
	0.4	0.49 ± 0.11	10
	0.04	0.043 ± 0.020	10
	0.004	-	-
Captan	200.	290. ± 290.	8
	20.	38. ± 16.	11
DEF	150.	270. ± 90.	7
	15.	24. ± 10.	9
	1.5	1.9 ± 1.2	11
	0.15	-	-
Propanil (Stam F-34)	800.	2,000. ± 600.	9
	80.	88. ± 46.	10
2,4-D acid	10,000.	-	-
	1,000.	-	-
Trifluralin	150.	590. ± 210.	11
	15.	47. ± 24.	9

* Mean ± one standard deviation.

Salinity during the exposure period averaged 32.4 °/₀₀ (22.9 to 34.0 °/₀₀), temperature was 12.6°C (11.0° to 15.0°C), the dissolved oxygen was 8.2 mg/liter (6.8 to 8.5 mg/liter) and the pH was 7.9 (7.0 to 8.3). Crabs were fed portions of starry flounder, Platichthys stellatus, or English sole, Parophrys vetulus, every other day, and uneaten food was removed after 24 hr. A photoperiod of 11 hr of light and 13 hr of darkness was used throughout.

In a second series of adult chronic bioassays, lasting 90 days, four concentrations each of carbofuran, chlordane and malathion were tested (Table 9). The numbers of crabs per concentration and in the control groups were the same as in the preceding tests. During these tests, which were run during the late fall and early winter, the salinity occasionally dropped to low levels during low tide periods not exceeding 5 hr. The salinities, as measured, averaged 25.4 °/₀₀ (11.4 to 32.7 °/₀₀), the temperatures averaged 10.0°C (6.6° to 11.8°C), and the dissolved oxygen and pH levels and the photoperiod were the same as in the preceding tests.

TABLE 9. PESTICIDE CONCENTRATIONS IN SEAWATER DURING ADULT CHRONIC EXPERIMENTS

Pesticide	Nominal concentration (μg/liter)	Measured concentration (μg/liter)	Number of analyses
Carbofuran	250.	210. ± 100.*	6
	25.	23. ± 14.	9
	2.5	1.7 ± 0.7	7
	0.25	-	-
Chlordane	100.	130. ± 30.	10
	10.	13. ± 4.	15
	1.	1.1 ± 0.5	15
	0.1	0.14 ± 0.12	15
Malathion	1,500.	2,400. ± 1,600.	2
	150.	180. ± 40.	16
	15.	15. ± 5.	14
	1.5	1.2 ± 0.5	15
Methoxychlor	40.	75. ± 36.	7
	4.	7.4 ± 2.4	7
	0.4	0.73 ± 0.21	10
	0.04	0.053 ± 0.028	7
Captan	200.	340. ± 40.	10
	20.	19. ± 4.	10
DEF	2,000.	2,600. ± 1,500.	7
	200.	380. ± 140.	10
	20.	35. ± 14.	10
	2.	3.0 ± 0.7	10
Propanil (Stam F-34)	4,000.	6,600. ± 3,100.	9
	400.	590. ± 170.	9
	40.	34. ± 42.	10
2,4-D acid	10,000.	-	-
	1,000.	-	-
Trifluralin	100.	300. ± 110.	9
	10.	33. ± 10.	10
	1.	2.6 ± 0.8	9

* Mean ± one standard deviation.

UPTAKE, LOSS, AND TISSUE DISTRIBUTION OF METHOXYCHLOR

Rates of uptake of methoxychlor by second and third instar juvenile crabs exposed to 0.04 and 2.0 μg/liter of pesticide were determined by analyzing subsamples consisting of 10 juvenile crabs each at 3 to 6 day intervals over an 18-day period. Adult crabs, 165 g and 107 mm in mean weight and carapace width, were exposed for 15 days to 1.8 and 7.5 μg/liter of methoxychlor, and individuals were analyzed for whole-body pesticide residues periodically during the exposure period and also during a 15-day depuration period. In addition, 10 adult crabs were taken from each exposure concentration at the end of the 15-day exposure period for the analysis of residues in specific tissues. The tissues from each group of 10 crabs were pooled for analysis. The measured methoxychlor concentration in each tissue, times the mean weight of each tissue for a 165 g crab, gave the total amount of pesticide in each tissue and this value divided by the sum of total, calculated methoxychlor from all tissues was used to calculate the percentage of total whole-body methoxychlor found in each tissue. The mean wet weight of each tissue was determined by dissecting and weighing the tissues from eight unexposed crabs. The weight of the blood was estimated as the difference between the whole-body weight and the sum of individual tissue weights.

Water conditions during these tests with both juveniles and adults were: temperature, 11.0°C (9.0° to 11.5°C); salinity, 31.0 °/₀₀ (30.2 to 32.7 °/₀₀); dissolved oxygen, 9.0 mg/liter (7.8 to 9.4 mg/liter); and pH, 7.9 (7.0 to 8.3). All other maintenance and exposure conditions were as described previously for the chronic toxicity experiments.

To determine whole-body residues in crabs after death from exposure to methoxychlor, adults, exposed to 1.8, 7.5, 18 and 32 μg/liter for up to 15 days under the conditions described previously, were removed from the water and analyzed for pesticide residues within 12 hr of death. Unless indicated otherwise, residue data are based on the wet weights of the crabs and their various tissues.

SALINITY TOLERANCE EXPERIMENT

In salinity tolerance tests with adult *C. magister*, crabs were pre-exposed in flowing water aquaria for 10 days to 10 μg/liter methoxychlor or acetone only. During this period the temperatures ranged from 12° to 14°C, the salinities from 30 to 33 °/₀₀ and dissolved oxygen was maintained at air saturation by vigorous aeration. The crabs were then transferred to glass troughs containing various seawater dilutions and 10 μg/liter methoxychlor or acetone supplied in a flowing bioassay system, and deaths were recorded daily during a 7-day exposure period. Temperature and dissolved oxygen were the same as during the pre-bioassay period. Frequent analysis of the seawater in these and later tests showed that the methoxychlor concentration was maintained in the flowing water systems to within ± 10% of the desired value.

OSMOTIC - IONIC REGULATION EXPERIMENTS

In order to examine the effects of methoxychlor on osmotic and ionic regulation, adult crabs were pre-exposed for 14 days to 10 µg/liter of methoxychlor or to acetone alone, as described previously for the salinity tolerance experiment. The crabs were then transferred to the same glass troughs used in the salinity tolerance experiment and exposed for 48 hr to various seawater dilutions with continued pesticide exposure. After 48 hr, blood and urine was collected for determination of the osmotic concentrations and the concentrations of Na^+, K^+, and Mg^{++} ions. Following the 14 day exposure period, another group of crabs was taken for the determination of gill ATPase activities.

Blood was obtained by puncture of the thin membrane at the base of the second or third walking leg by Pasteur pipettes, transferred to polyethylene vials, and frozen for storage. Upon thawing, the clots were broken up with a glass stirring rod and aliquots of serum were used undiluted for measurements of osmotic concentration or after dilution for the analysis of Na^+, K^+, and Mg^{++} ions. Urine was collected into 0.25 ml syringes by careful insertion of a blunted 24-gauge needle into the nephropore, and was stored frozen in small polyethylene vials. Osmotic concentrations were determined with a Wescor Model 5100 vapor pressure osmometer. A Perkin-Elmer Model 403 atomic absorption spectrophotometer was used in the determination of Na^+, K^+, and Mg^{++} ions.

ATPASE ASSAY

ATPase activity was determined in whole homogenates of *C. magister* gill. Gills from freshly killed crabs were homogenized in 0.05 M glycylglycine buffer, pH 7.4, at high speed in a Sorvall omnimixer. The whole homogenate was then centrifuged for 10 min at 30 x g to sediment coarse materials. Total ATPase activity was measured in a 2-ml reaction medium containing 100 mM NaCl, 15 mM KCL, 5 mM $MgCl_2$, 6 mM ATP and 50 mM glycylglycine buffer, pH 7.4. In some assays, Mg ATPase was determined by adding 1 mM ouabain to the reaction medium to inhibit the NaK activated enzyme. NaK ATPase was calculated as the difference between total ATPase and Mg ATPase. The reaction was allowed to proceed for 30 min at 15°C and was terminated by pipetting 0.5 ml aliquots of the reaction medium into 0.5 ml 10% TCA. Phosphate was determined by the method of Fiske and SubbaRow (1925). Protein was estimated by the Lowry procedure (Lowry et al., 1951). Specific activity was expressed as µmoles P_i hydrolyzed/mg protein/hr.

SECTION 5

RESULTS AND DISCUSSION

THE FUNGICIDE CAPTAN

Captan is reported to have a short half-life in aqueous solution; the rate of its hydrolysis to tetrahydrophthalimide and tetrahydrophthalic acid is related directly to pH and temperature (J. N. Osperson and D. E. Pack, Chevron Chemical Company, Richmond, Calif., personal communication cited by Hermanutz et al., 1973). Hermanutz et al. (1973) reported that the half-life of captan in Lake Superior water at a pH of 7.6 is about 7 hr at 12°C and about 1 hr at 25°C. Since data are lacking on the half-life of captan in seawater, we examined the rate of its breakdown at several concentrations under conditions prevailing in the acute toxicity bioassays. After 24 hr, the concentrations of captan remaining in seawater were from 48 to 74% of the initial concentrations, which ranged from 23 to 2,300 µg/liter (Table 10).

TABLE 10. DECAY RATE OF CAPTAN IN SEAWATER OF 25 °/₀₀ SALINITY AT 13°C *

Initial measured concentration (µg/liter)	Measured concentration after 24 hr (µg/liter)	Percent of initial concentration after 24 hr	Estimated half-life (hr)
2,300	1,100	48	23
1,400	900	64	37
300	200	67	41
220	150	68	43
23	17	74	54

* pH ranged from 7.6 to 7.9.

The half-life of captan estimated from these data ranged from 23 to 54 hr.

The effects of captan on egg hatching success, on success of molting from prezoeae to first stage zoeae, and on the activity of first stage zoeae, determined at the end of a 24-hr exposure period, are summarized in Figure 4.

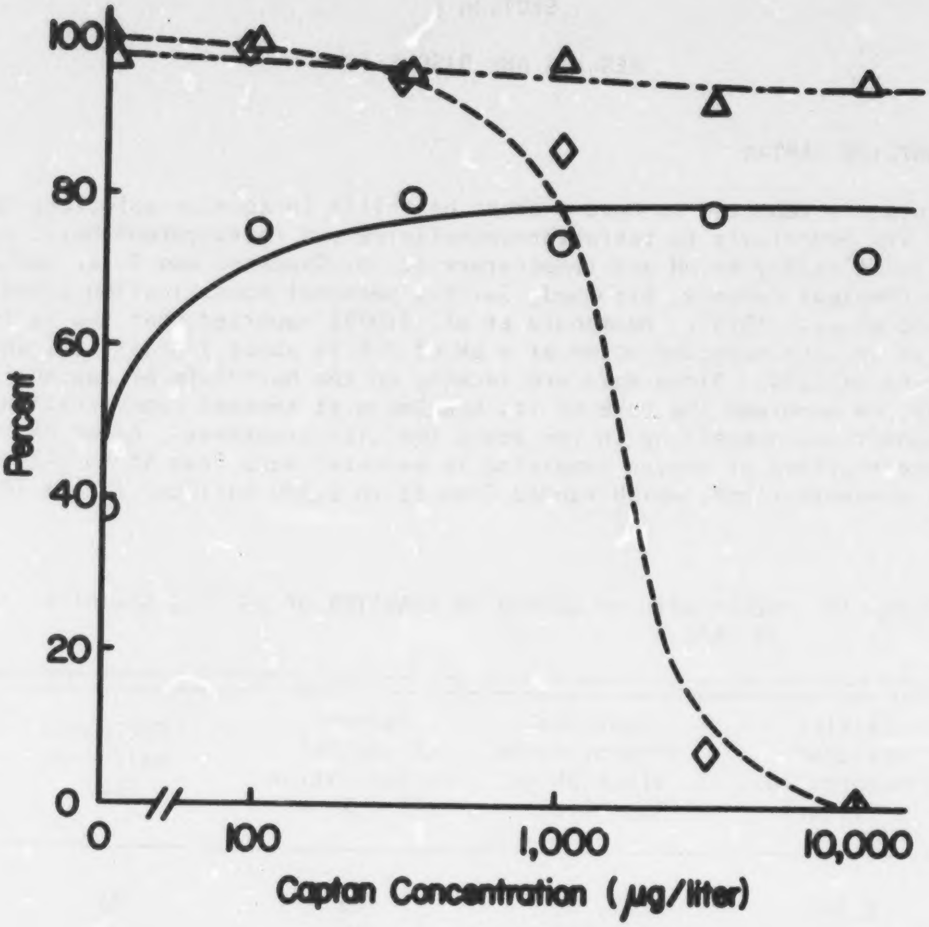


Figure 4. Percent hatch of Dungeness crab eggs (○——○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—·—△), and percent of developed first stage zoeae which are motile (◇----◇) as a function of captan concentration during a 24 hr exposure period.

The mean and standard deviations of hatching successes in both seawater containing acetone (shown simply as zero captan concentration in Figure 4) and untreated seawater controls, in replicate tests, were 39 ± 7% and 36 ± 16% of the initial numbers of eggs, respectively. Hatching success in the presence of all concentrations of captan tested exceeded this value, averaging 76 ± 7% in ten separate cultures; these results are similar to those reported previously by Buchanan et al. (1970) for eggs exposed to the pesticide Sevin. Of those prezoeae hatching, more than 90% molted successfully to first stage zoeae even at 10,000 µg/liter, the highest captan concentration tested. The developed first stage zoeae, however, were largely immobilized at only 3,300 µg/liter. A morphological abnormality was also observed in the zoeae developing at 3,300 and 10,000 µg/liter of captan. The abnormality was a lateral enlargement of the newly formed carapace following the prezoeal molt to a width about twice that found for the control larvae.

In 96-hr acute tests with first stage zoeae, the concentrations causing 50% immobilization of zoeae (EC50) in 24, 48 and 96 hr were 5,600, 3,500 and 1,500 µg/liter of technical captan, respectively (Table 11). A 50% wettable powder formulation of captan appeared to be slightly more toxic in a comparable series of tests; the 24-, 48- and 96-hr EC50s were 3,200, 700 and 360 µg/liter, respectively. The 96-hr LC50 for technical captan exceeded 10,000 µg/liter, but the 96-hr LC50 for the 50% wettable formulation was 8,000 µg/liter (Table 11). In 96-hr acute toxicity tests with the juvenile and adult stages of the crabs, we did not observe any deaths at the highest concentrations tested, which were 10,000 and 100,000 µg/liter, respectively. At these highest concentrations of technical captan, the solubility of the fungicide in seawater had clearly been exceeded, since we observed the formation of a heavy white precipitate. The solubility of technical captan in water is reported to be 3,300 µg/liter at 25°C (Chevron Chemical Company, Ortho Division, San Francisco, Calif. Orthocide® Experimental Data Sheet, April, 1970). In seawater at 20°C, the solubility has been estimated to be 3,500 µg/liter (G.W. Newell, Stanford Research Institute, Palo Alto, Calif., personal communication).

First stage zoeae exposed to 200 µg/liter captan were rapidly killed in the chronic toxicity tests. In the first experiment lasting only 18 days, the time to 50% mortality at this exposure concentration was 6 days, and that to 100% mortality was 10 days (Figure 5). In the second test, the time to 50% mortality was about 9 days, and total mortalities had occurred by day 21 (Figure 6). In the latter test, none of the zoeae exposed to 200 µg/liter captan molted successfully to second stage zoeae, even though some of these larvae survived beyond the first molt period, which occurred between days 12 and 16 in control groups (Figure 7). Zoeae exposed to only 20 µg/liter captan and less survived as well as controls until the termination of the two tests on days 18 and 69, respectively. In the longer test, survival at these lower fungicide concentrations and in the control cultures still exceeded 80% of the original numbers of zoeae by day 50, at which time molting to the fifth zoeal stage was occurring. After this period, however, the mortality rates of all of the treatment groups, including the controls, increased greatly (Figure 6). Exposure of zoeae to 2 and 20 µg/liter of captan did not appear to affect the duration of the first two zoeal stages, but by the time of the third zoeal molt, larvae exposed to both of the fungicide concentrations showed a molting delay of about 3 days when compared with controls (Figure 7). A delay was no longer apparent, however, in the 2 µg/liter group by the time of the fourth molt.

The first chronic toxicity test with juvenile crabs was continued for a period of 36 days; at the end, most of the crabs had molted to the second instar, and survival in controls remained above 80% of the original number of crabs. At this time, survival of crabs exposed to the highest captan concentration, 200 µg/liter, was as high as that of the controls (Figure 8). Furthermore, exposure of juvenile crabs to captan in these tests did not appear to delay the initiation of the first molt (Figure 9).

TABLE 11. ACUTE TOXICITY OF CAPTAN TO FIRST INSTAR ZOEAE, FIRST INSTAR JUVENILE CRABS, AND ADULT CRABS

Stage of crab	Pesticide formulation	Toxic concentrations (µg/liter)					
		24-hr		48-hr		96-hr	
		EC50	LC50	EC50	LC50	EC50	LC50
Zoeae	Technical	5,600	>10,000	3,500	>10,000	1,500	>10,000
	50% wettable	3,200	>10,000	700	>10,000	360	8,000
Juvenile	Technical	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Adult	Technical	-	>100,000	-	>100,000	-	>100,000

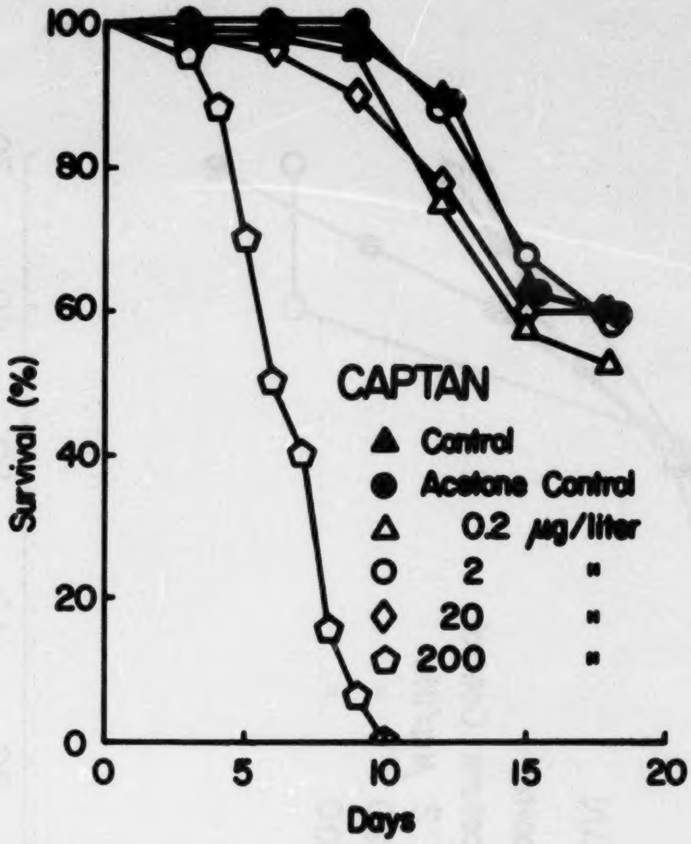


Figure 5. Effect of continuous exposure to captan on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973.

In a second chronic exposure test with juveniles, the crabs were exposed only to 20 and 200 µg/liter captan. These crabs had been reared in the laboratory in uncontaminated seawater for 2 months following their metamorphosis from the magalopae stage and were mostly third instars at the time the test was initiated. The mortality rates of crabs exposed to the fungicide appeared to be higher than those of the controls during the initial month, but by the end of the test on day 80, the survival of the fungicide-exposed crabs was as high as that of the acetone-exposed control crabs (Figure 10).

Adult crabs exposed to 20 and 200 µg/liter captan suffered no mortalities over a 75-day exposure period (Figure 11). Also, there was no apparent effect of the fungicide on the behavior or the feeding activity of either juvenile or adult crabs.

In acute toxicity tests, Dungeness crab zoeae exhibited a lesser sensi-

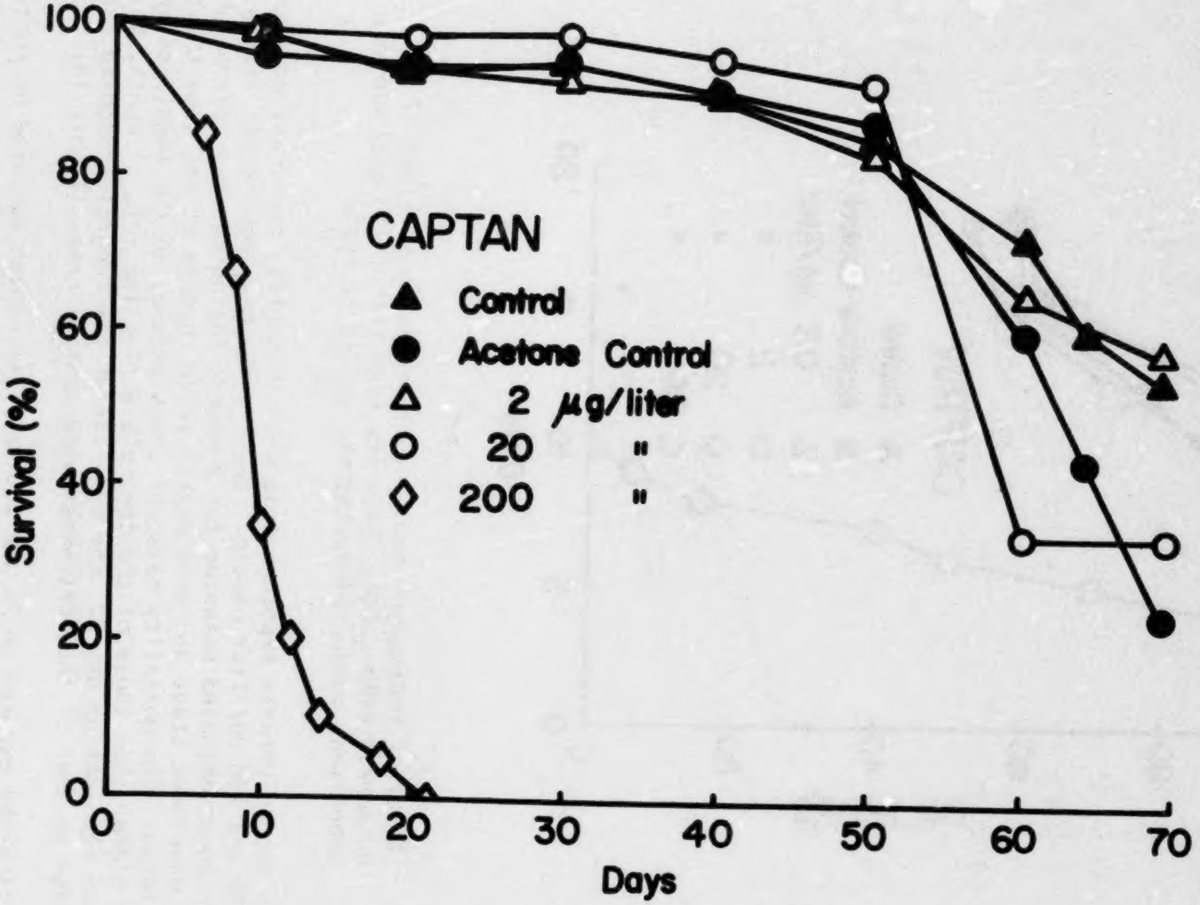


Figure 6. Effect of continuous exposure to captan on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

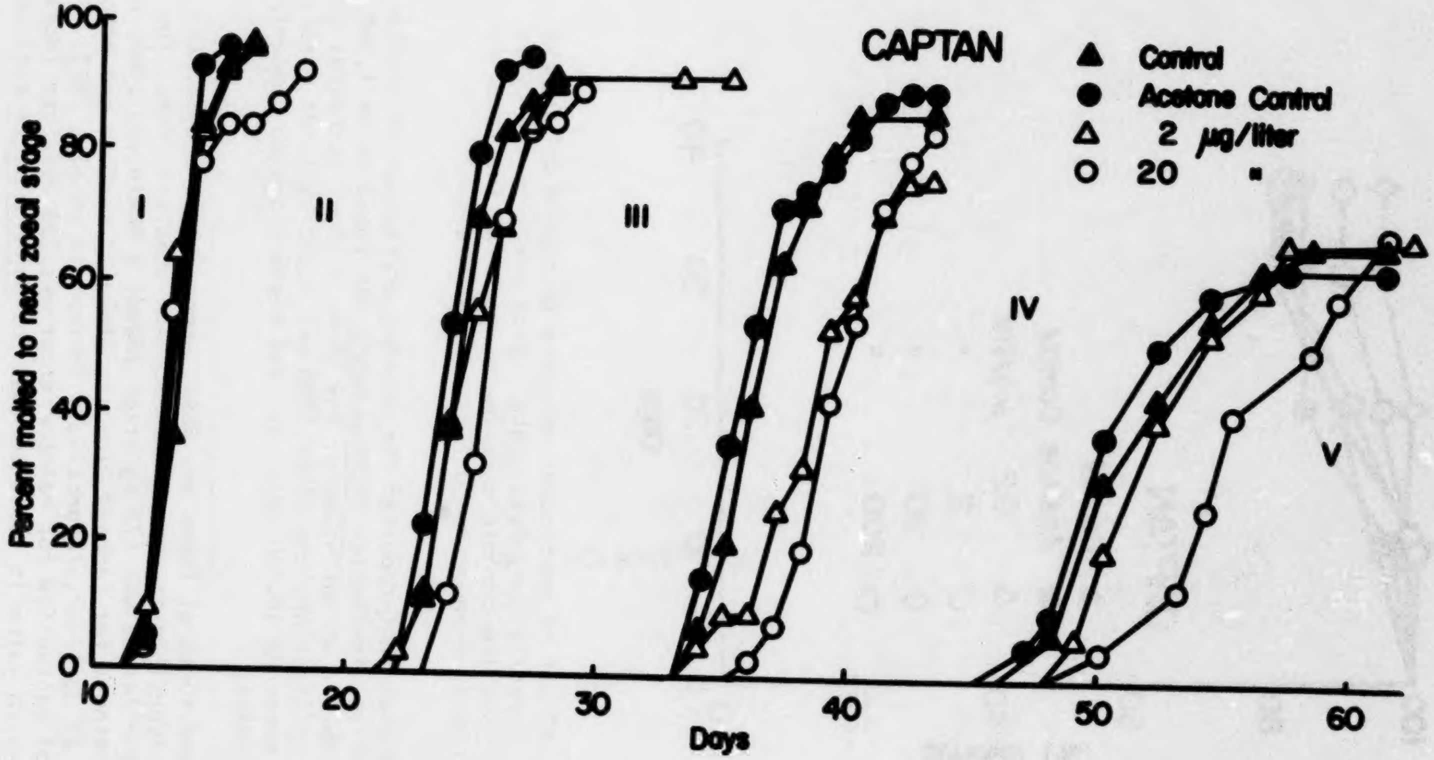


Figure 7. Effect of continuous exposure to captan on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success for each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

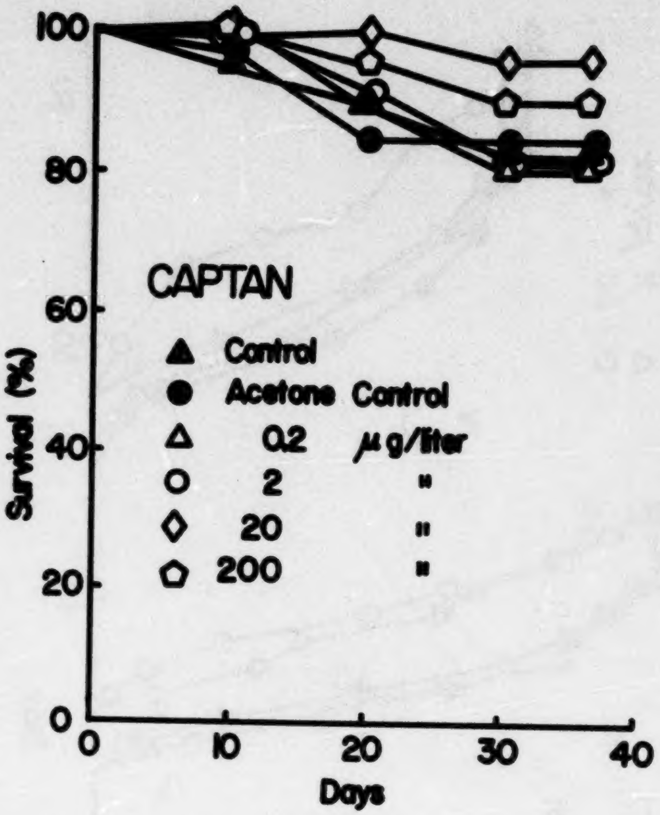


Figure 8. Effect of continuous exposure to captan on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs.

tivity to captan than that reported for another crustacean of similar size. The 26-hr LC50 of the freshwater Daphnia magna was found to be 1,300 μg/liter (Frear & Boyd, 1967). In our studies, the 24-hr EC50 of technical captan for zoeae was 5,600 μg/liter and the 96-hr EC50 was 1,500 μg/liter (Table 11). LC50 values all exceeded 10,000 μg/liter, the highest captan concentration employed in the tests.

Our data, and those of Frear and Boyd, suggest that crustaceans may be slightly less susceptible to captan intoxication than are fish. The 90-min LC50 for zebrafish larvae was 670 μg/liter (Abedi & McKinley, 1967) and the acute lethal threshold for fathead minnows, bluegill sunfish and brook trout were 64, 72 and 29 μg/liter, respectively (Hermanutz et al., 1973). However, the 72-hr LC50 of captan-50W for rainbow trout was 320 μg/liter (Holland et al., 1960), which value is close to that for Daphnia magna estimated by

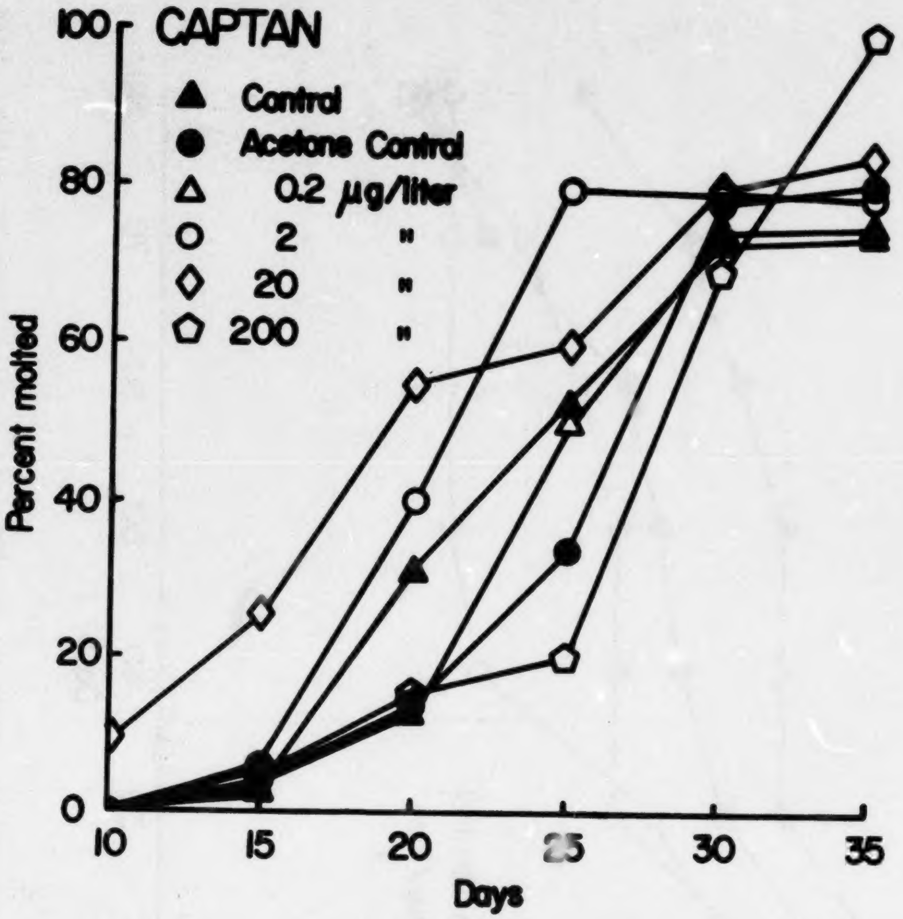


Figure 9. Effect of continuous exposure to captan on molting of first instar Dungeness crabs to the second instar. The molt success is expressed as the percentage of the original number of first instar crabs.

extrapolation of the data of Frear and Boyd to 72 hr.

Of all the crab life history stages examined, the zoeal stage was the most sensitive to captan intoxication in both acute and chronic toxicity tests. In the chronic exposure experiments, survival of this stage appeared unaffected at 20 µg/liter of captan but was clearly reduced at 200 µg/liter (Figures 5 & 6). In addition, at 20 µg/liter, there was an indication that the molting of later stages of crab larvae was delayed, although the results were somewhat equivocal (Figure 7). In similar, long-term experiments with juvenile and adult crabs, both of these stages were unaffected by continuous exposure to 200 µg/liter, the highest concentration tested. The same relationship between age of _C. magister_ and sensitivity to the carbamate

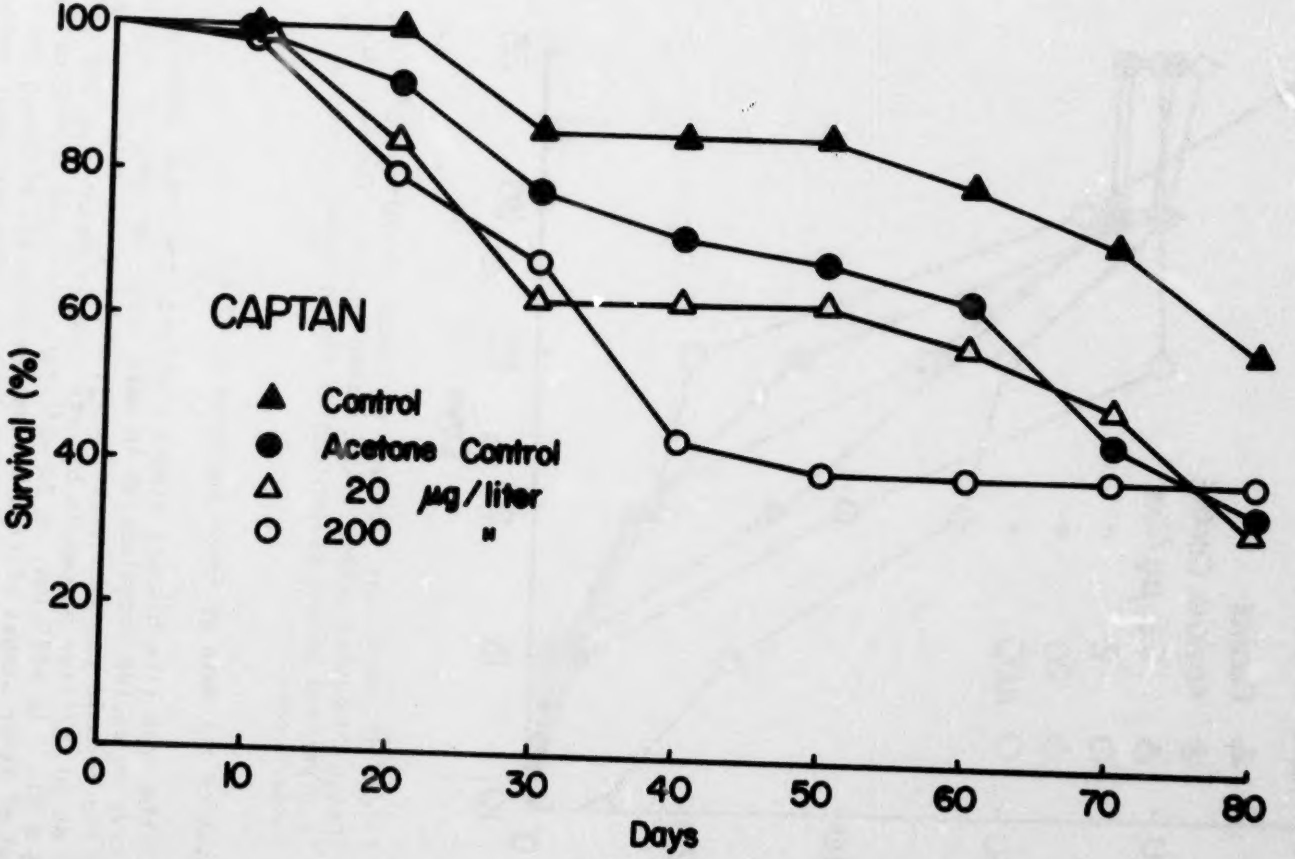


Figure 10. Effect of continuous exposure to captan on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs.

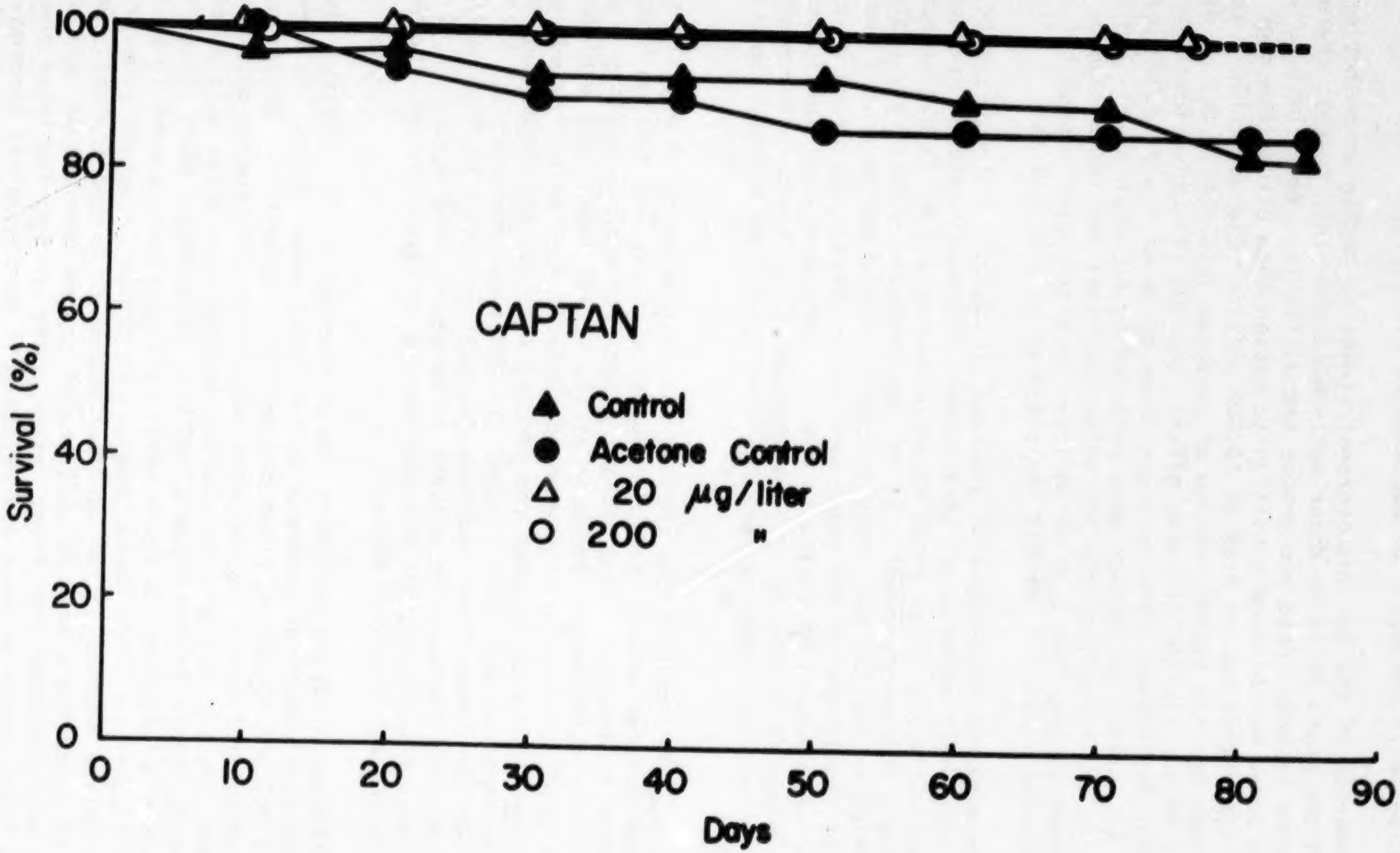


Figure 11. Effect of continuous exposure to captan on survival of adult Dungeness crabs.

insecticide Sevin was noted by Buchanan et al. (1970).

Exposures of the egg and prezoeal stages to captan were shorter than those in the acute toxicity experiments with the older stages; therefore, comparisons between these age groups are difficult. Nevertheless, it appears that the zoeae may be more sensitive to captan than either the eggs or prezoeae. Concentrations as high as 10,000 µg/liter did not inhibit the hatching of eggs nor the transformation of prezoeae into zoeae, but by using activity as a criterion of toxic affect, the 50% effective level for those zoeae that developed in the test was found to be as low as 2,000 µg/liter (Figure 4). Similar results were obtained by Buchanan et al. (1970) with Sevin. In their studies, the 50% effective level for inhibition of prezoeal development ranged from 6 to 30 µg/liter in three experiments, but activity of developing zoeae was largely inhibited at only 5 µg/liter.

The apparent resistance of prezoeae to captan is probably attributable to the very short duration of this stage. The newly hatched prezoeae normally molt into the first zoeal stage within only 5 to 15 min of hatching (Buchanan & Millemann, 1969). If the egg membrane effectively resists penetration of captan, the newly hatched prezoeae may have very low tissue levels of the fungicide and these may not be greatly increased by a further 5 to 15 min exposure to captan in water. Subsequent additional accumulation of the fungicide by the newly metamorphosed first instar zoeae might then result in impaired swimming behavior attributable to a higher body burden of captan.

We are uncertain of the reasons for the low hatch of eggs in the seawater and acetone control groups compared with the 76% average hatch found in all captan treatments. In a similar experiment with C. magister eggs exposed to the insecticide Sevin, Buchanan et al. (1970) found that a higher hatching success also occurred in eggs exposed to Sevin than in untreated eggs, although the difference between the treated eggs and untreated eggs was not as great as reported here with captan. In addition, Buchanan and Millemann (1969) earlier showed that reducing the salinity from 30 °/₀₀ to 15 °/₀₀ nearly doubled the hatching success of Dungeness crab eggs in a 36-hr period. These results indicate that the application of stressful conditions may accelerate the egg hatching process.

Although a captan concentration as high as 10,000 µg/liter did not prevent the development of prezoeae to the first zoeal stage, the zoeae produced at 3,300 and 10,000 µg/liter did exhibit a deformity. Zoeae developing at these concentrations of captan were seen to have a lateral enlargement of the carapace. We did not observe similar deformations after molting of zoeae exposed to captan in the chronic toxicity experiments even though the highest concentration employed in those tests, 200 µg/liter, proved to be lethal within 21 days to first instar zoeae. It should be noted, however, that none of the larvae exposed to 200 µg/liter molted to second instars. Zoeae exposed chronically to only 20 µg/liter of captan survived as well as control larvae and developed from first instar zoeae through the later zoeal stages to the fifth instar without any evidence of morphological abnormality. Production of the zoeal deformity apparently requires exposure of molting zoeae to a higher concentration of captan than used in the chronic tests, but such concentrations can only be survived by zoeae in short-term exposures.

Interestingly, a gross morphological malformation has been observed in at least one other aquatic species exposed to captan. Abedi and McKinley (1967) reported a deformity to the head in larvae of zebrafish Brachydanio rerio, acutely exposed to captan concentrations of 500 to 1,000 µg/liter. The injury was always associated with the death of the fish larvae. A common characteristic of the captan-caused morphological lesions in both zebrafish larvae and crab larvae is an enlargement or swelling of the affected region.

Captan is chemically similar to the teratogenic compound thalidomide. This fact has lead several investigators, in studies with vertebrates, to compare the teratogenic potential of captan and other N-substituted phthalimide compounds with thalidomide. The results, however, are inconclusive. Captan did not produce thalidomide-like embryopathic effects in two studies with rabbits (Fabro et al., 1965; Kennedy et al., 1968), but did in two later investigations, one with rabbits (McLaughlin et al., 1969) and the other with chicks (Verrett et al., 1969).

It is tempting to suggest that the abnormalities observed in this study with crab larvae and in studies with vertebrates, including those with fish larvae (Abedi and McKinley, 1967), have a common etiology. Unfortunately, the reasons for the abnormal development are not clearly understood. Further, it is known that captan interacts with both soluble and insoluble thiol groups in biological material (Owens and Blaak, 1960b; Richmond and Somers, 1966) and is thus likely to have broad-spectrum effects on biological systems. Such effects have been shown to include increased mutagenesis in E. coli and inhibition of mitosis and DNA synthesis and increased incidence of chromosome breaks in mammalian cell cultures (Legator et al., 1969). Also, captan interferes with oxidative [illegible]horylation and mitochondrial structure (Nelson, 1971a; 1971b) and with [illegible] synthesis from acetate and oxaloacetate (Owens and Blaak, 1960a)

The results of our work [illegible] indicate that the environmental consequences for the use of capt[illegible] marine or estuarine waters are likely to be minimal. Even direct appl[illegible]ion of the fungicide at the manufacturer's highest recommended rates (10-12 lb/acre for Orthocide-50W) to an estuary of 2 meters average depth would result in an average seawater concentration of only 336 µg/liter. If maintained, this concentration would probably be toxic to crab zoeae within a few days, but because of the high rate of degradation of the fungicide in seawater, the concentration resulting from a single application could be expected to rapidly fall below the levels that have demonstratable chronic toxicity.

Although the morphological effects of captan, observed during the transformation of prezoeae to zoeae are of considerable interest in connection with the question of the teratogenic potential of captan, the ecological significance of this effect is probably minimal. It did not occur when eggs were exposed to captan concentrations lower than those that result in death of first instar zoeae during 96-hr acute exposures. Whether longer exposure of eggs would or would not modify this response is unknown.

THE INSECTICIDE METHOXYCHLOR

Toxicity

Methoxychlor exposures of up to 10 μg/liter did not reduce the percentage of eggs that were able to hatch during a 24-hr period. In fact, as was noted with captan, an increase in hatching rate of all pesticide-exposed eggs was noted over that found in the controls (Figure 12). Larvae from nearly

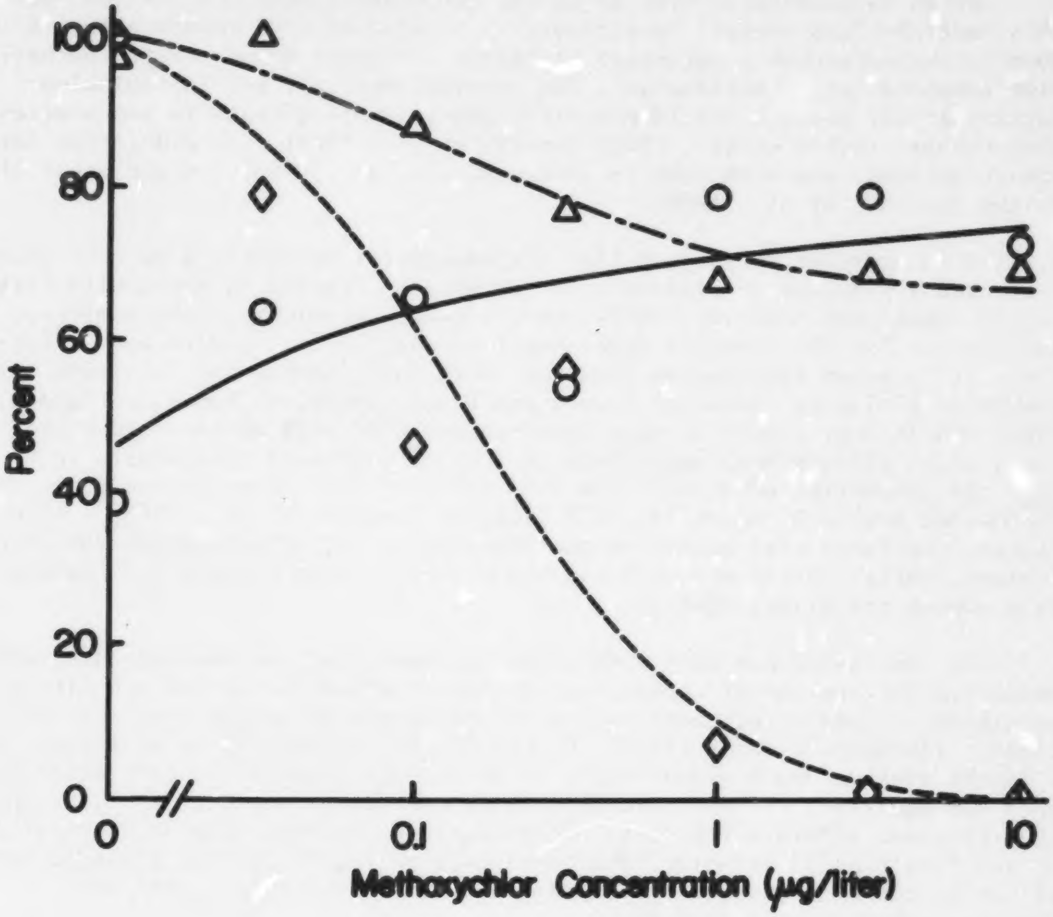


Figure 12. Percent hatch of Dungeness crab eggs (○——○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—·—△), and percent of developed first stage zoeae which are motile (◇----◇) as a function of methoxychlor concentration during a 24-hr exposure period.

all of the eggs that hatched in the control vessels, successfully completed development through the brief prezoeal stage and were first instar zoeae by the end of 24 hr. At increasingly higher concentrations of methoxychlor, the successful completion of this developmental sequence decreased until at 1 to 10 µg/liter only 70% of hatching eggs became first instar zoeae (Figure 12). Of the zoeae that developed, a reduction in motility of 50 and 90% occurred at methoxychlor concentrations of about 0.18 and 1.0 µg/liter, respectively, whereas 100% of the controls were motile (Figure 12).

Acute toxicity of methoxychlor to later developmental stages was inversely related to crab age. The 96-hr LC50s for zoeae, juveniles and adults were 0.42, 5.10 and 130 µg/liter, respectively (Table 12). The latter value exceeds the estimated solubility of methoxychlor of 50 µg/liter in seawater of 25 °/₀₀ salinity at 13°C and is based on the amount of methoxychlor added to the bioassay containers. A similar relationship between developmental stage and methoxychlor sensitivity was found by using sublethal criteria with zoeae and juvenile crabs (Table 12).

TABLE 12. ACUTE TOXICITY OF METHOXYCHLOR TO FIRST INSTAR ZOEAE, FIRST INSTAR JUVENILE CRABS, AND ADULT CRABS

Developmental stage	Toxic concentrations (µg/liter)					
	24-hr		48-hr		96-hr	
	EC50	LC50	EC50	LC50	EC50	LC50
Zoeae	0.50	>0.92	0.23	0.92	0.05	0.42
Juvenile	7.20	12.0	5.50	8.20	5.10	5.10
Adult	-	>920	-	>920	-	130

In the initial chronic experiment with zoeae, of 18 days duration, methoxychlor did not affect the survival of larvae even at 0.1 µg/liter, the highest concentration employed (Figure 13). In the second experiment, zoeae were reared in the presence of methoxychlor for 69 days by which time they had reached the fifth zoeal stage of development. Survival of controls and of zoeae exposed to the lowest test concentration of 0.005 µg/liter exceeded 85% for 50 days (Figure 14). Thereafter, survival of zoeae in all groups, including the controls decreased markedly, possibly due to increased incidence of bacterial infection or to a nutritional deficiency. Of the larvae exposed to 0.5 µg/liter, 50% were dead by day 5 and all were dead by day 24. Survival of larvae exposed to the intermediate concentration of 0.05 µg/liter was slightly less than that found in the controls, and all were dead by day 65. This group, in addition, experienced a slight delay of the onset of molting at each zoeal stage (Figure 15). Survivors of the 0.5 µg/liter methoxychlor exposure also were delayed in molting to the second zoeal stage.

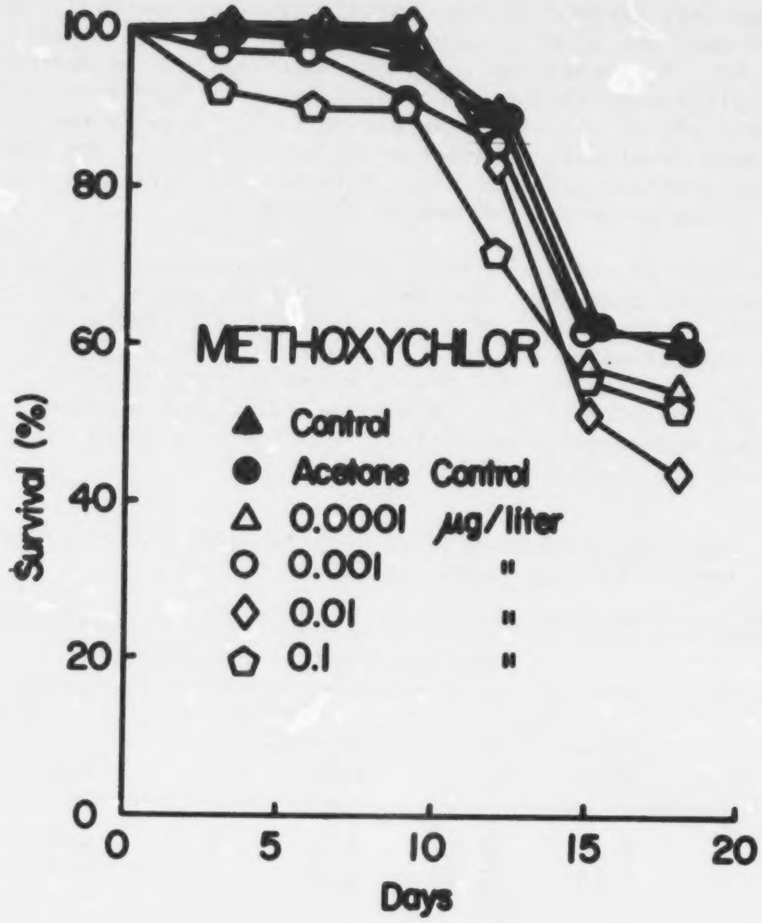


Figure 13. Effect of continuous exposure to methoxychlor on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973.

Juvenile crabs were markedly affected by chronic exposures to 0.4 and 4.0 µg/liter of methoxychlor. During the first test, lasting 36 days, survival of crabs at these concentrations was similar to that of the controls until day 20, but declined rapidly thereafter (Figure 16). In the second test, which lasted for 80 days, 50% of the crabs exposed to these same concentrations had died by days 27 and 12, respectively, and all were dead by days 80 and 40 (Figure 17). Survival of crabs exposed to the two lowest concentrations in the second test (0.04 and 0.004 µg/liter) was similar to that of controls and averaged 76% by day 60. Thereafter, there was a gradual decline in the survival of these crabs; but the relationship of survival of the exposed crabs to that of the controls did not change (Figure 17).

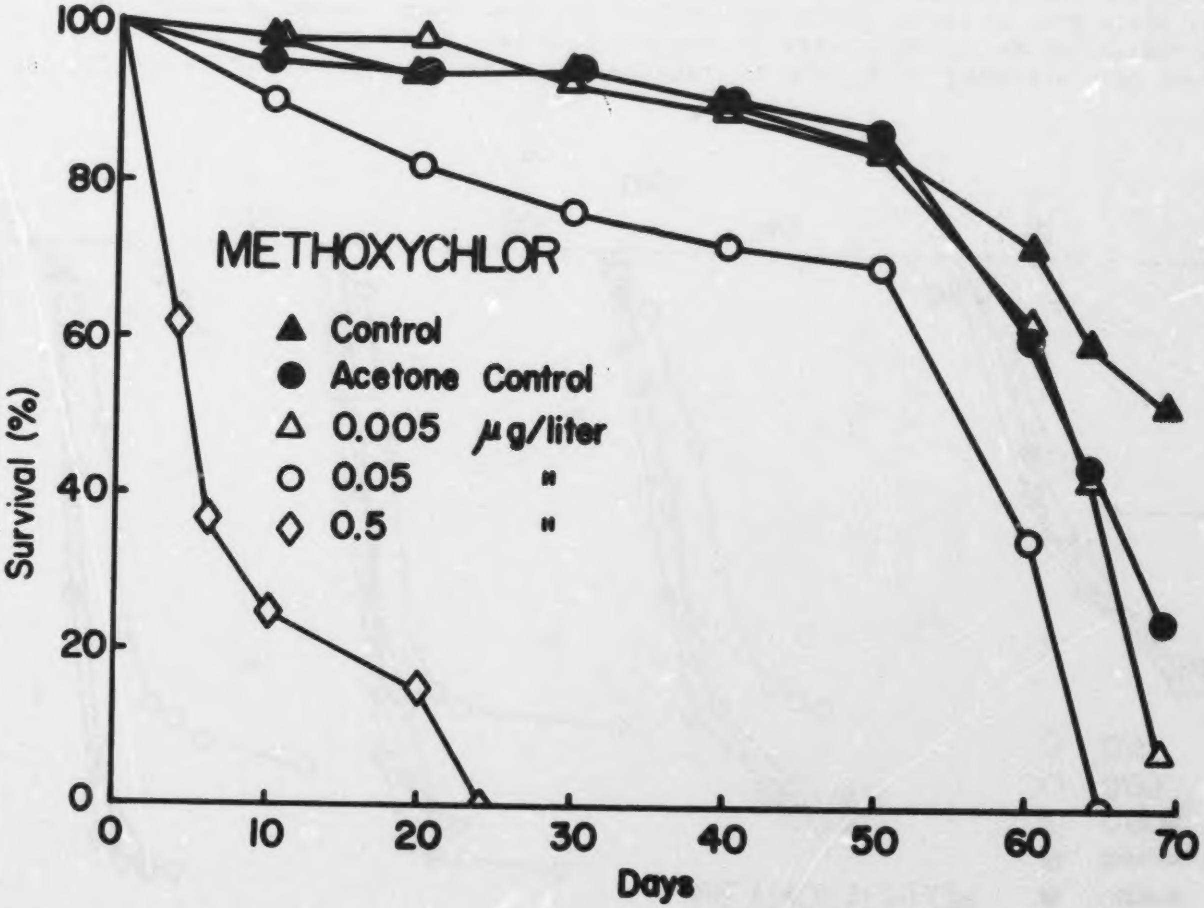


Figure 14. Effect of continuous exposure to methoxychlor on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

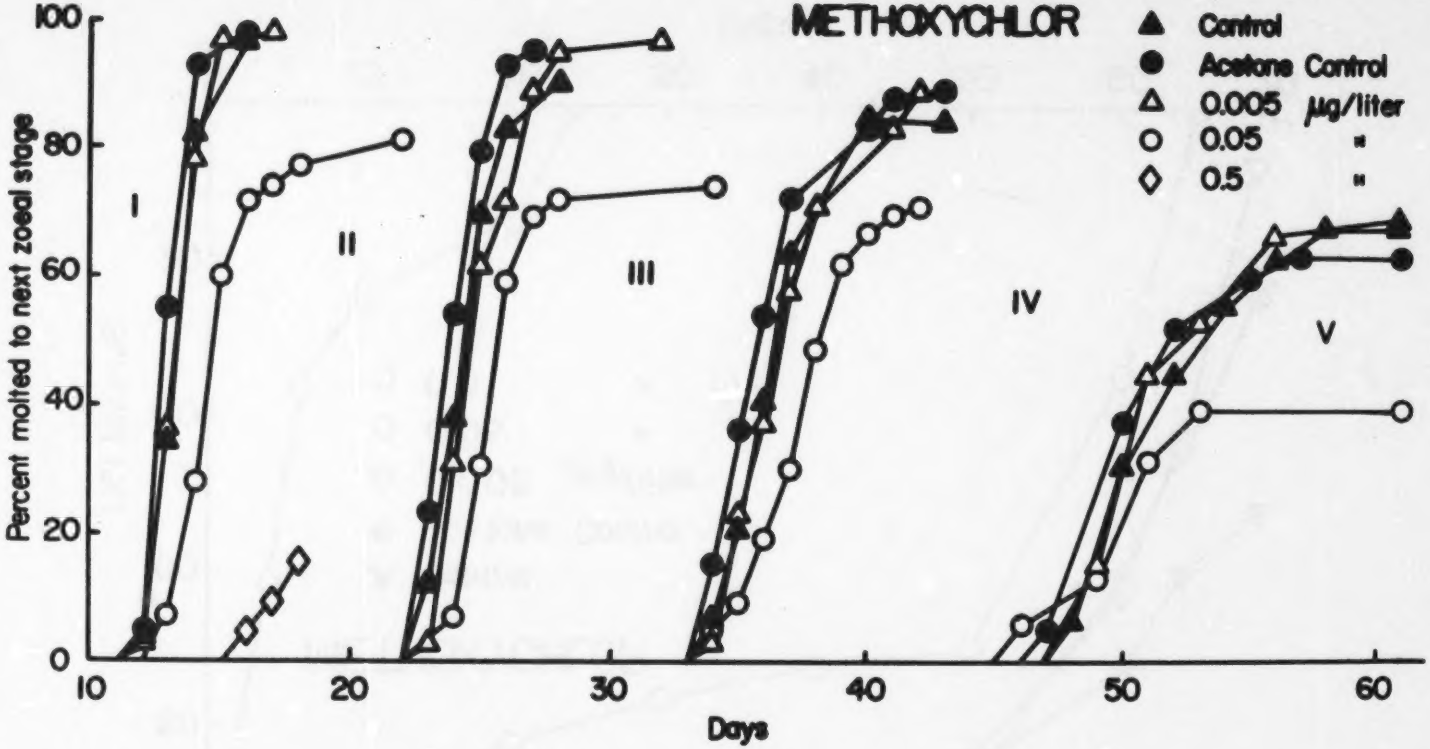


Figure 15. Effect of continuous exposure to methoxychlor on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success for each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

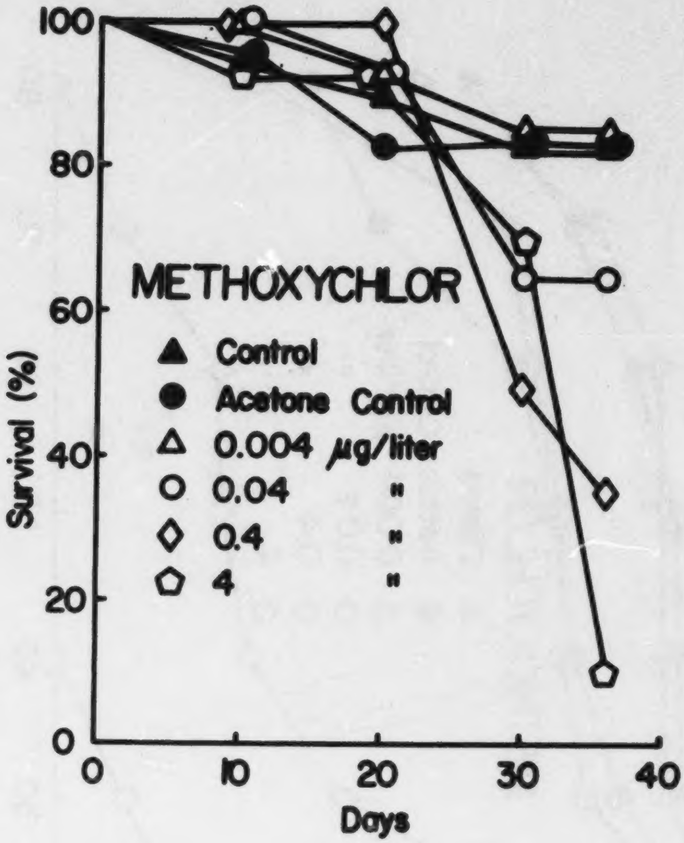


Figure 16. Effect of continuous exposure to methoxychlor on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs.

Sensitivity of juvenile crabs to methoxychlor was greatest during molting or shortly thereafter. Of second and third instar crabs molting at 0.4 and 4.0 µg/liter, 38% and 100%, respectively, died within 72 hr after ecdysis in the 80-day test (Table 13). Deaths of crabs at these same concentrations in the 36-day tests also appeared to occur at, or slightly following, the period of molting (Figures 16 and 18).

Methoxychlor also delayed the onset of molting of crabs in the 36-day test (Figure 18). The controls began to molt to second instars on about day 15, and by day 30 this molt was essentially complete. Molting was delayed in all crabs exposed to methoxychlor, but this effect was most extreme among crabs exposed to 4.0 µg/liter. We were unable to unequivocally measure possible effects of methoxychlor on molting of juveniles in the second,

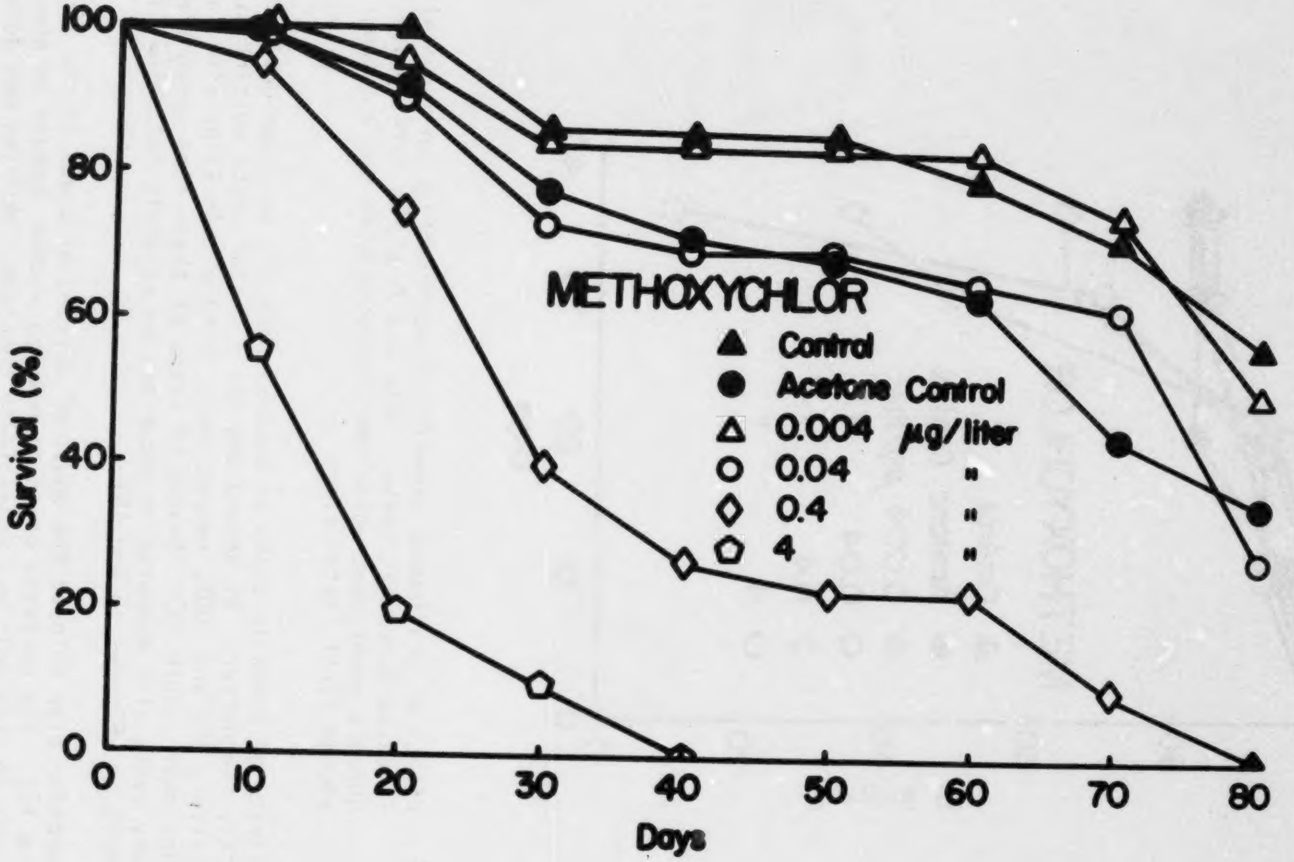


Figure 17. Effect of continuous exposure to methoxychlor on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs.

TABLE 13. EFFECT OF METHOXYCHLOR ON SURVIVAL OF MOLTED JUVENILE AND ADULT CRABS DURING CONTINUOUS EXPOSURE TO THE PESTICIDE FOR 80 AND 85 DAYS, RESPECTIVELY

Methoxychlor concentration (μg/liter)	Original number of crabs per treatment	Percentage of crabs which molted during experiment	Percentage of molting crabs that died within 72 hr (juveniles) or 24 hr (adults) after molting
Juvenile crabs			
Control	54	100	18
Acetone control	54	81	19
0.004	18	100	0
0.04	18	83	18
0.4	18	89	38
4.0	18	83	100
Adult crabs			
Control	30	43	0
Acetone control	30	67	0
0.04	10	70	14
0.4	10	80	0
4.0	10	60	100
40.0	10	20	100

80-day, test because molting of these older crabs was largely out of synchrony by this time. However, some crabs in the control groups and each concentration of methoxychlor in this test had molted by day 7 of exposure, and approximately 75% of crabs in all groups had molted by day 19 so that no evidence for an effect of the pesticide on molting was evident in this experiment.

The increase in size of methoxychlor-exposed juvenile crabs that molted during the 80-day test was less than that of the controls. The mean carapace widths of fourth instar crabs in control and 4.0 μg/liter groups were 19.1 mm and 16.6 mm, respectively, or greater by 34% and 16% than the mean width of third instar crabs (14.3 mm). These differences were found to be statistically significant ($\alpha = 0.01$) by the method of least significant differences.

All adult crabs exposed to 40.0 μg/liter of methoxychlor had died by day 47, and by the end of the test period, 60% of those exposed to 4.0 μg/liter were dead also (Figure 19). Survival of controls and of crabs exposed to the two lower concentrations of 0.4 and 0.04 μg/liter was about 85% at the test's end. As was true of the juvenile crabs, sensitivity of the adults to methoxychlor increased during ecdysis. None of the controls that molted

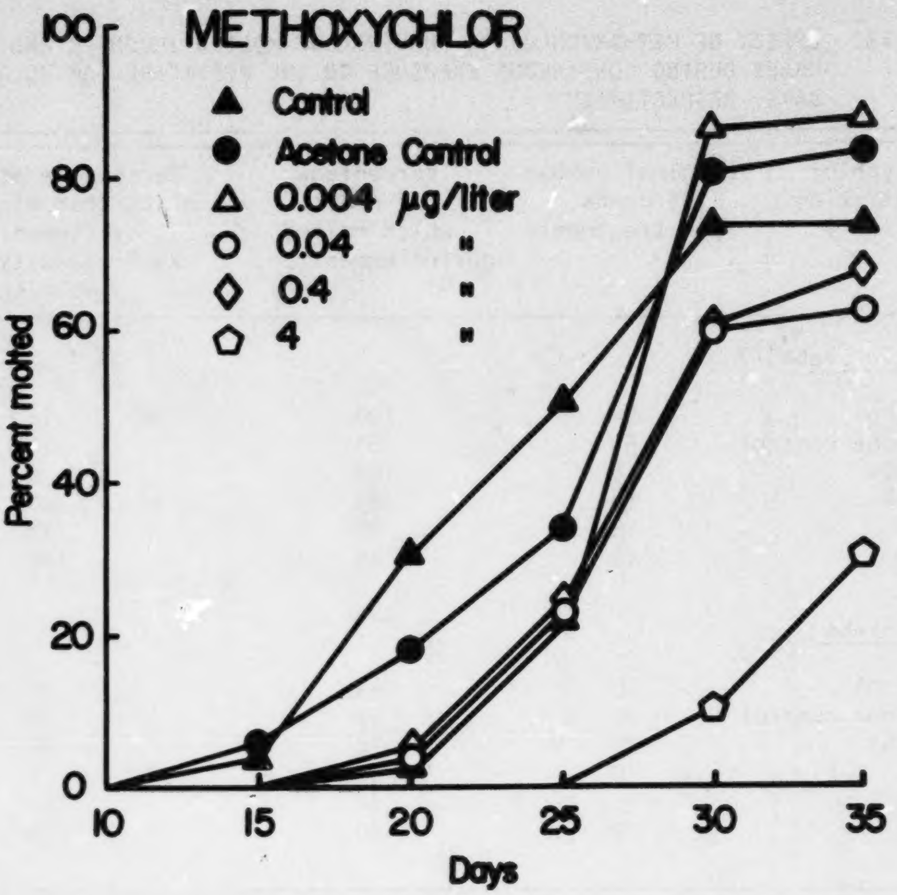


Figure 18. Effect of continuous exposure to methoxychlor on molting of first instar Dungeness crabs to the second instar. The molt success is expressed as the percentage of the original number of first instar crabs.

during the 85-day test died. However, 100% of the crabs that molted during exposure to 4.0 and 40.0 μg/liter died within 24 hr (Table 13).

Adult crabs affected by methoxychlor were hyperactive and moved their mouth parts and chelipeds much more frequently than did the control animals. The more severely affected crabs were unable to maintain an upright posture and some remained flipped on their dorsal surface for several weeks, until death. Feeding was active, except during the molting period, among control crabs and those exposed to the two lower concentrations of methoxychlor, but crabs exposed to 40 μg/liter did not eat after test days 6 to 8. Crabs exposed to 4.0 μg/liter had difficulty in locating food, and would usually shred and scatter it rather than consume it entirely.

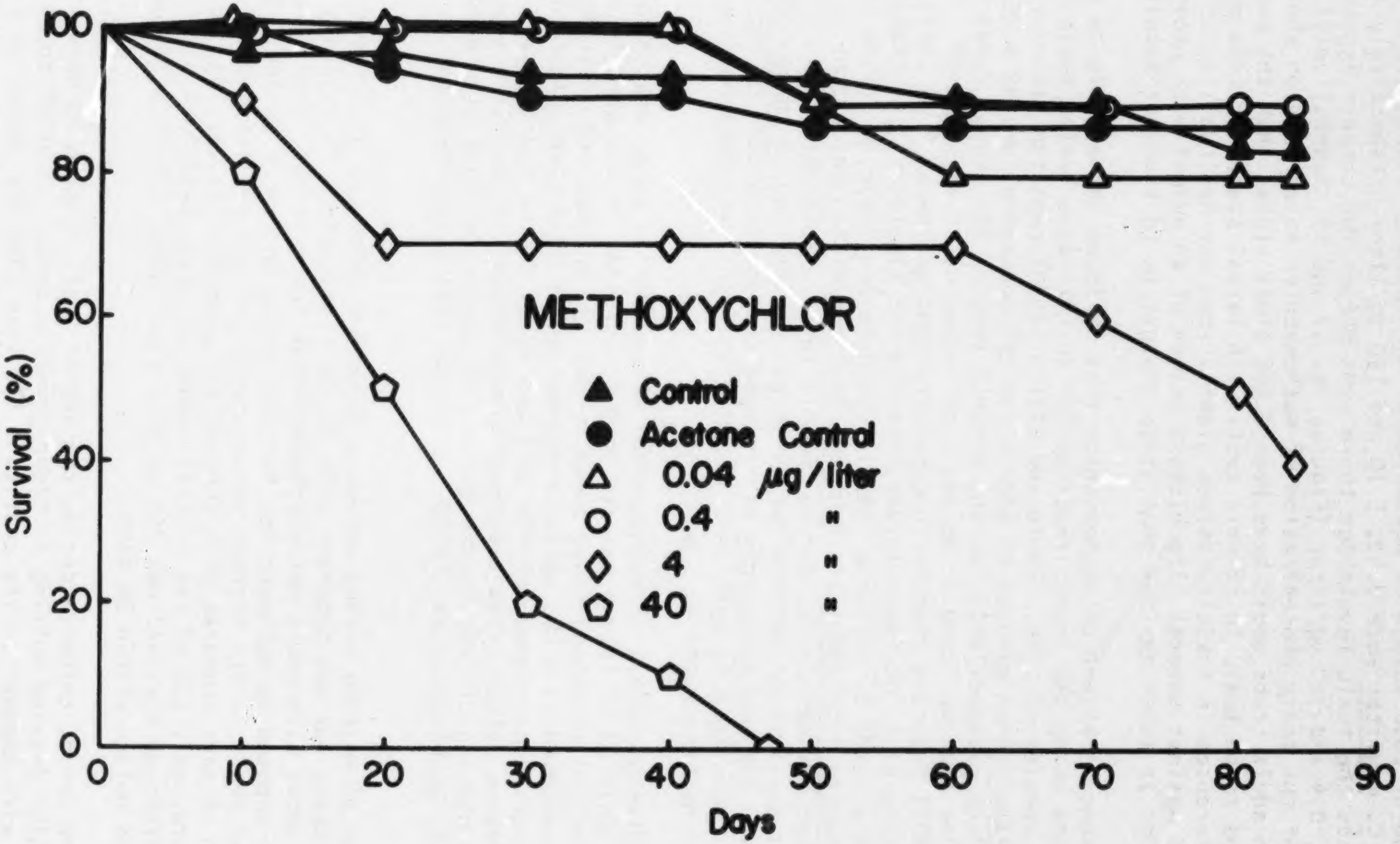


Figure 19. Effect of continuous exposure to methoxychlor on survival of adult Dungeness crabs.

The 96-hr LC50 values of methoxychlor for larval, juvenile, and adult stages of C. magister were 0.42, 5.10 and 130 µg/liter, respectively (Table 12), whereas the toxic levels for these same stages for chronic exposure were only 0.05, 0.4 and 4.0 µg/liter (Figures 14, 17 and 19, respectively). An estimate of the toxic concentration of methoxychlor based only on short-term tests with adult crabs would have been 2,600 times higher than the concentrations found to be toxic in chronic tests with larval stages of this animal. These differences in toxicity values clearly show the desirability of testing pesticides against several life history stages of an animal with prolonged exposure, or at least against that stage thought to be the most sensitive.

The concentrations of methoxychlor that we found to be toxic to C. magister are among the lowest reported for insecticides tested against this and other species of crab. Poole and Willis (1970) reported high mortalities of C. magister zoeae exposed to endrin and DDT concentrations of 0.25 and 0.50 µg/liter, respectively, during chronic tests. The lowest level of methoxychlor that we found to be toxic to larvae was 0.05 µg/liter. Buchanan et al. (1970) reported that Sevin concentrations as low as 0.32 µg/liter delayed molting of C. magister larvae, but did not significantly affect survival. In a study by Epifanio (1971), dieldrin was toxic to larvae of the crabs Leptodius floridanus and Panopeus herbstii at concentrations of 1.0 and 5.0 µg/liter, respectively, during chronic tests. Bookhout et al. (1972) found that survival of various larval stages of the crabs Rithropanopeus harrisii and Menippe mercenaria was reduced in concentrations of the insecticide mirex down to 0.01 µg/liter; in this instance lower than our toxicity values for methoxychlor.

A sublethal effect of methoxychlor observed with zoeae was delay of molting. Only 7% of larvae exposed to 0.05 µg/liter in the chronic experiment had molted to second stage zoeae by day 13, whereas 46% of the controls and those exposed to 0.005 µg/liter had molted by this time (Figure 15). This same trend continued for the next two molts to the fourth zoeal stage, but was not as evident when treatment groups molted to the fifth stage. Delays in molting of crab larvae exposed to pesticides have been previously observed by Buchanan et al. (1970), Epifanio (1971), and Bookhout et al. (1972).

Delay of molting during exposure to methoxychlor was also noted with juvenile crabs but was apparent only in the first test. This 36-day experiment was begun with newly metamorphosed first instar crabs, that were subsequently exposed to methoxychlor for the entire intermolt period. Onset of molting of crabs in the highest concentration of 4.0 µg/liter was delayed by more than 10 days compared with that of the controls (Figure 18). By day 30 of exposure, only 10% of the initial number of crabs held in 4.0 µg/liter had molted, although survival was 70% at this time; about 77% of both control groups had molted within 30 days.

There was no evidence during the second test, lasting 80 days, that methoxychlor delayed molting in older juvenile crabs. Most of these crabs probably were exposed to the pesticide for less than the latter half of their intermolt phase, suggesting that the effect of methoxychlor on molting may depend upon the length or timing of exposure of the crabs during an intermolt period.

A possible explanation for a relationship between duration of intermolt exposure to methoxychlor and incidence of molt delay is related to the neuroendocrine control of molting. The interrelationship of the X- and Y-organs in crustacean ecdysal processes has been well documented (Passano, 1960; Lockwood, 1967). The neurosecretory X-organ-sinus gland complex produces a molt-inhibiting hormone that acts directly on the Y-organ to depress synthesis or release of its molting hormone. At the time in the molt cycle when inhibition ceases, the Y-organ releases the molting hormone, and proecdysis is initiated and quickly becomes irreversible (Lockwood, 1967). If methoxychlor affected the X-organ in such a way that production or release of the molt-inhibiting hormone continued beyond the normal time, the effect would be to delay initiation of the molting process. Another possibility is that methoxychlor directly delayed the production or release of molting hormone by the Y-organ, but this hypothesis is less attractive because methoxychlor is believed to be a neurotoxin (O'Brien, 1967), and molting hormone production by the Y-organ is not a neurosecretory process (Passano, 1960).

The hypothesis that action of methoxychlor on the X-organ affects molting may not explain molting delays observed with C. magister larvae exposed to 0.05 μg/liter. Costlow (1966) has produced evidence that the control of molting in larval crustaceans may not involve X-organ inhibition in some instances, because eyestalk ablation in Rhithropanopeus zoeae did not result in anticipated acceleration of molting.

Another possible cause of delay of molting may be starvation of crabs (Passano, 1960). We observed that larval and juvenile crabs exposed to methoxychlor did eat, but we did not make quantitative determinations of food consumption during the tests. The amount eaten or the efficiency of food utilization may have been reduced by the high pesticide concentrations and molting thereby delayed.

There was an apparent increase in sensitivity of the crabs to methoxychlor during or shortly after ecdysis. A similar observation has been reported by Duke et al. (1970) and Nimmo et al. (1971) for crustaceans exposed to the PCB, Aroclor 1254. All of the juvenile crabs that molted during exposure to 4.0 μg/liter of methoxychlor died within 72 hr after ecdysis (Table 13). Similarly, 100% of the adult crabs that molted in 40.0 and 4.0 μg/liter died within 24 hr after ecdysis. Bookhout et al. (1972) suggested that release of stored mirex from fat reserves could account for increased deaths of crab larvae during molting to megalopae. A similar release of methoxychlor from the lipid storage sites of our exposed adult and juvenile crabs may explain their increased sensitivity at ecdysis. Hepatopancreatic lipid reserves are highest as proecdysis is initiated, and levels decline through molting until active feeding is resumed (Passano, 1960).

Possibly, increases in blood volume during ecdysis contributed to higher circulating levels of methoxychlor at this time, a result that could lead to higher levels of the pesticide being transported to affected tissues. The blood volume of Cancer can increase nine-fold during ecdysis (Passano, 1960) due to active uptake of water through the gut (Lockwood, 1967). Additionally, blood levels of lipids rise during ecdysis (Passano, 1960), and this also could increase the carrying capacity of the blood for lipid soluble pesticides such as methoxychlor.

Finally, the increased death rate of methoxychlor exposed crabs after molting may occur because pesticides are more readily absorbed by the animal at this time. The integument of newly molted crabs is thinner and in all probability more permeable to the pesticide than after extensive mineralization has occurred. If this hypothesis is correct, then one would not anticipate an increase in death rate of zoeae during the molting periods because even during intermolt of the larval stages, the exoskeleton is not thick or heavily mineralized. In fact, we noted that the mortality of zoeae which was attributable to methoxychlor exposure was constant throughout the exposure period and was not apparently correlated with the time of molting (Figures 14 and 15).

Uptake, Loss, and Tissue Distribution of Methoxychlor

Although the range of pesticide residue concentrations in adult crabs was wide, there was a tendency for animals killed at the highest methoxychlor concentrations to have higher whole body residues than did those killed at the lower concentrations (Table 14). Crabs killed after exposure to 32.0 µg/liter had an average methoxychlor concentration of 1.59 mg/kg in whole-body tissues, whereas crabs killed after exposure to 7.5 µg/liter had whole-body residues averaging only 0.57 mg/kg. Most of the crabs killed after exposure to the lower concentrations had recently molted, whereas none of the animals exposed to 32.0 µg/liter molted (Table 14). The higher residue levels in the latter group may reflect greater quantities of pesticide absorbed to, or otherwise associated with, the exoskeleton and its epiflora,

TABLE 14. WHOLE BODY METHOXYCHLOR CONCENTRATIONS IN ADULT CRABS KILLED DURING CONTINUOUS EXPOSURE TO METHOXYCHLOR FOR UP TO 15 DAYS

Methoxychlor concentration in exposure water (µg/liter)	Number of animals analyzed	Whole body concentration of methoxychlor (mg/kg)*	Percentage of killed crabs which had just molted
1.8	1	0.11	100
7.5	5	0.57 (0.46 - 1.3)	80
18.0	6	0.95 (0.45 - 1.3)	67
32.0	14	1.59 (1.10 - 2.5)	0

---

* Mean concentration with range in parentheses.

rather than a significant difference in the levels of pesticide residing in the affected internal tissues.

We estimated the amount of methoxychlor which associated loosely with the exoskeleton and its epiflora, by measuring the whole-body residues in two groups of crabs; one having received the routine washing with water only, and the second having also been thoroughly rinsed with acetone. Whole-body residues in crabs exposed for 72 hr to 7.5 µg/liter were 39% greater when acetone was not used as a rinse than when it was (Table 15). Microscopic examination of crabs washed only with water revealed the presence of diatoms still on the exoskeleton. It is probable that much of the pesticide removed from crabs rinsed with acetone had been associated with these microorganisms.

TABLE 15. WHOLE BODY RESIDUES OF METHOXYCHLOR IN ADULT CRABS WASHED WITH WATER ONLY OR WITH WATER FIRST, FOLLOWED BY ACETONE BEFORE PESTICIDE ANALYSIS*

Exposure period (hr)	Whole body residues (mg/kg)†	
	Water-washed crabs	Water + acetone-washed crabs
12	0.08	0.07
24	0.15	0.10
48	0.19	0.12
72	0.23	0.14

* Crabs were exposed for up to 3 days to a pesticide concentration in seawater of 7.5 µg/liter.

† Each value is the methoxychlor level in a pooled sample of five crabs.

Uptake of methoxychlor by juvenile crabs was linearly related to time, but not proportional to the exposure concentration (Figure 20). After 12 days, the whole-body pesticide concentrations in crabs exposed to 0.04 and 2.0 µg/liter were 0.11 and 0.88 mg/kg wet weight, respectively, and represented concentration factors of 2,725 and 440 above the exposure levels. After 12 days, many crabs exposed to the higher concentration had died, indicating that lethal whole-body levels of methoxychlor had been attained.

In contrast to the apparent zero order uptake curves for juvenile crabs, those for adults approximated first order kinetics (Figure 21). At 15 days, residues in crabs exposed to 1.8 and 7.5 µg/liter were about 0.11 and 0.48 mg/kg, respectively, with the uptake curves approaching plateaus at this time. The loss of methoxychlor was also a first order relationship, and only 5% to 6% of the residues present at day 15 remained by day 30, 15 days after the termination of methoxychlor exposures. The 12-day concentration factors for adult crabs exposed to 1.8 and 7.5 µg/liter were 58 and 60; this, unlike the situation with juvenile crabs, indicates that the uptake rate was

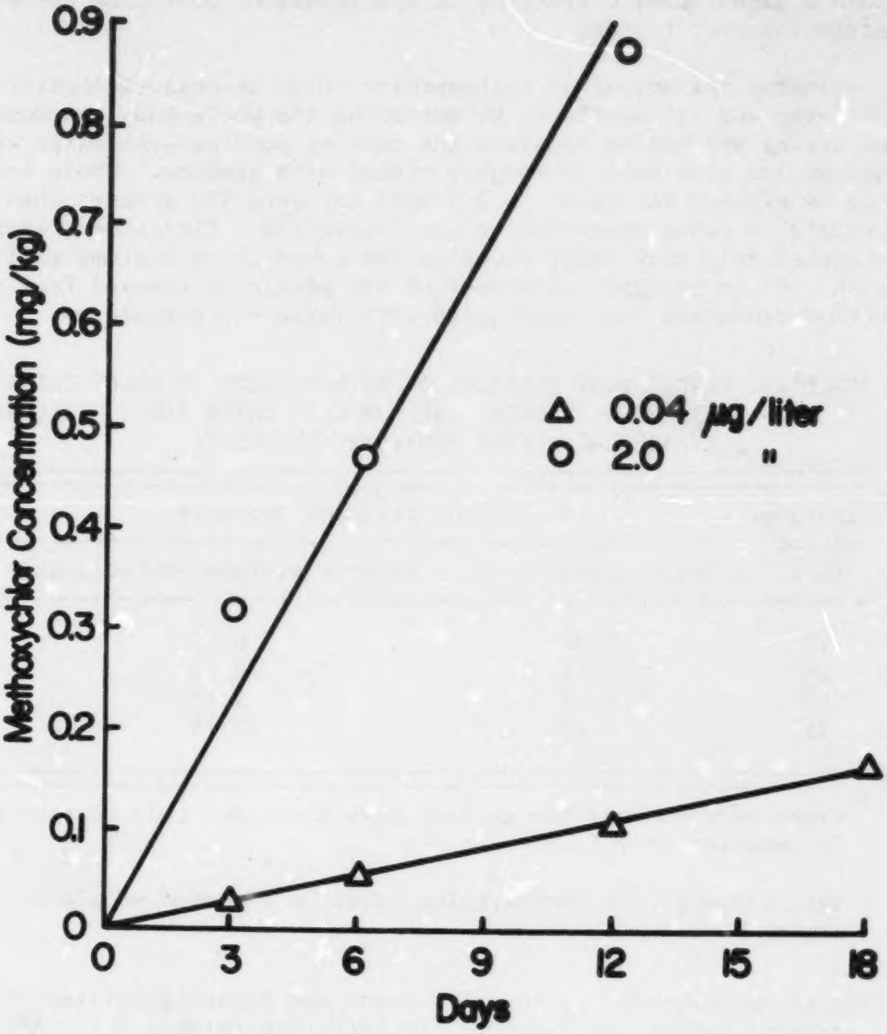


Figure 20. Whole body concentration of methoxychlor in juvenile crab, Cancer magister, as a function of time of exposure to the pesticide in seawater.

approximately proportional to the exposure concentration.

The highest concentrations of methoxychlor in individual tissues of exposed adult crabs were found in the exoskeleton, gill, and hepatopancreas, in decreasing order (Table 16). The concentration factors (tissue concentration/exposure concentration) for these three tissues in crabs exposed to 1.8 µg/liter were 183, 122, and 78, respectively, and for crabs exposed to 7.5 µg/liter were 89, 56, and 40. Dividing the sum of methoxychlor recovered in all tissues at each exposure concentration by the total weight of the crabs gives a whole-body residue of 0.16 mg/kg and 0.33 mg/kg for

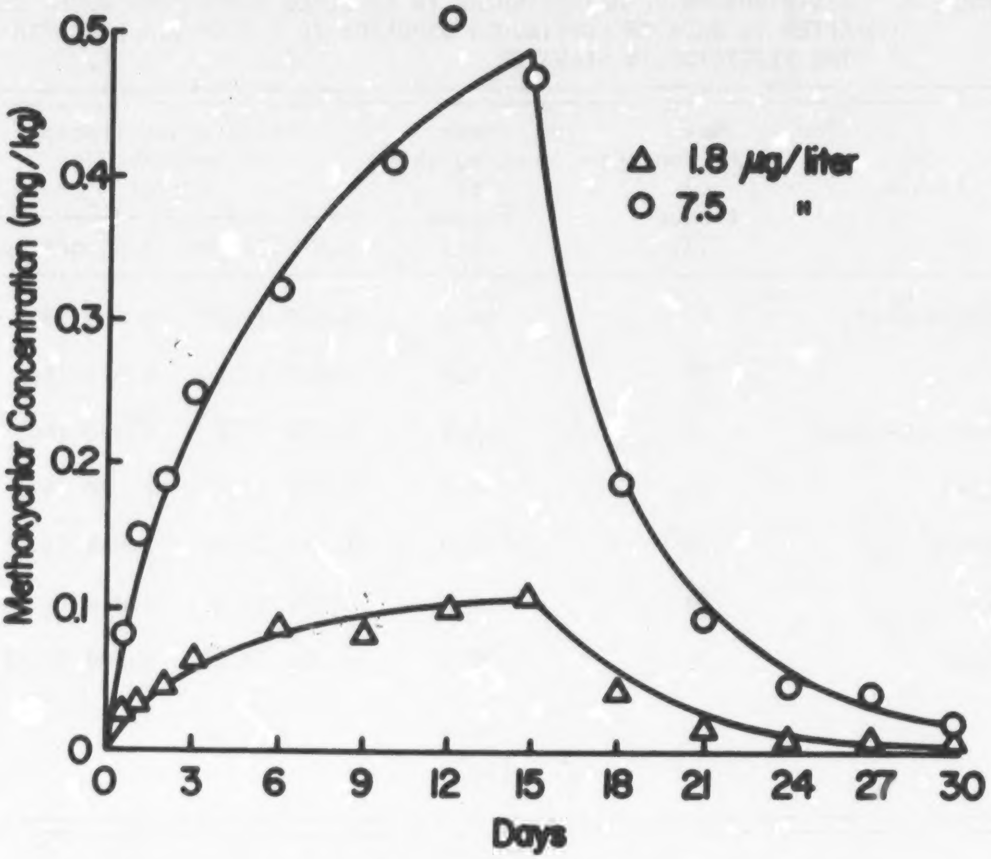


Figure 21. Whole body concentration of methoxychlor in adult crabs, Cancer magister, as a function of time during and after a 15-day exposure to the pesticide in seawater.

water exposures of 1.8 and 7.5 μg/liter, respectively, values which compare favorably to those determined in the experiments with whole crabs (Figure 21). The concentrations in the exoskeleton were about 83 times greater than those in the blood, which contained the lowest levels of all tissues assayed. The weight of the exoskeleton is about 39% of the wet weight of whole crabs, and about 81% of the total methoxychlor was calculated to have been present in this tissue at both water exposure levels (Table 16). Most of the remaining pesticide was found to be concentrated in the hepatopancreas and gill tissues. When the calculated tissue levels of methoxychlor were based on dry weights, the gills were found to have had the highest pesticide concentration, about three times higher than those in the exoskeleton.

There is little information on the uptake and loss of methoxychlor by aquatic invertebrates. Kapoor et al. (1970) found that levels of methoxychlor in larvae of the mosquito, Culex, and a snail were 0.48 and 15.7 mg/kg, respectively, after 7 and 23 days of exposure in a model ecosystem.

TABLE 16. DISTRIBUTION OF METHOXYCHLOR IN SELECTED TISSUES OF ADULT CRABS AFTER 15 DAYS OF CONTINUOUS EXPOSURE TO 1.8 OR 7.5 μg/LITER OF THE PESTICIDE IN SEAWATER

Tissue	Mean water content of tissue* (%)	Mean wet weight of tissue† (g)	Tissue concentration of methoxychlor (μg/g)	
			1.8 μg/liter	7.5 μg/liter
Exoskeleton	40	64.5	0.330 (183)#	0.670 (89)
Gill	88	5.9	0.220 (122)	0.420 (56)
Hepatopancreas	74	14.4	0.140 (78)	0.300 (40)
Gonad	60	6.0	0.070 (39)	0.320 (43)
Muscle	76	45.0	0.014 (7.7)	0.044 (5.9)
Heart	84	0.7	0.006 (3.3)	0.014 (1.9)
Blood	93	28.5	0.004 (2.2)	0.008 (1.1)
		165.0		

* Mean of 8 individual determinations.

† Based on a mean whole body weight of 165 g.

# Numbers in parentheses are Concentration Factors = $\frac{\text{TISSUE CONCENTRATION}}{\text{EXPOSURE CONCENTRATION}}$

Juvenile crabs exposed to 2.0 μg/liter had whole-body levels of 0.48 mg/kg after 6 days in our experiments. Uptake of the pesticide by Culex, however, was through their food rather than directly from water. Reinbold et al. (1971), using dry weight as a basis for estimating whole-body levels of pesticides, reported that Daphnia magna and snails, Physa sp., exposed to 3 μg/liter of methoxychlor for 3 days had tissue levels of 24 and 22 mg/kg, respectively; their levels represent concentration factors of 8,000 and 7,300. In our study, juvenile crabs exposed to 2.0 μg/liter of methoxychlor had whole-body levels of 0.3 mg/kg after 3 days of exposure. Even when corrected for dry weight, this value would only be 1.2 mg/kg, giving a concentration factor of 600. The relatively low accumulation of methoxychlor in C. magister suggests that this species may efficiently metabolize the pesticide or not absorb it as rapidly.

Concentration of methoxychlor in body tissues was more rapid in juvenile

TABLE 16. CONTINUED

Total of methoxychlor recovered (µg)		Percent of total recovery (%)	
1.8 µg/liter	7.5 µg/liter	1.8 µg/liter	7.5 µg/liter
21.3	43.2	82.6	79.9
1.30	2.48	5.0	4.6
2.02	4.32	7.8	8.0
0.42	1.92	1.6	3.5
0.63	1.98	2.4	3.7
0.004	0.010	0.02	0.02
0.11	0.23	0.43	0.43
25.8	54.1	99.9	100.2

crabs than in adults. Body size of the two stages may account for this difference in uptake rates. Juvenile and adult crabs averaged, respectively, 14 and 107 mm in width and 0.8 and 165 g in wet weight. Juvenile crabs exposed to 2.0 µg/liter methoxychlor for 12 days had whole-body levels of 0.88 mg/kg, whereas adults exposed to 1.8 µg/liter had whole-body levels of only 0.10 mg/kg by this same time.

Wildish and Zitko (1971) reported that the rate of uptake of Aroclor 1254 by an amphipod increased as the ratio of body or branchial surface area to body weight increased. If there is an inverse relationship between rate of methoxychlor accumulation in tissues and the size of crabs, then the larvae may have had the highest rate of pesticide uptake, although this was not measured, and this could explain the increasing sensitivity to methoxychlor we observed for successively smaller stages.

The highest concentrations of methoxychlor were in different tissues than have been reported in other crustacea. Nimmo et al. (1970) found that the hepatopancreas contained most of the DDT found in two species of shrimp; the levels being 200 times higher in that organ than in whole-body samples. The PCB, Aroclor 1254, was concentrated most in the hepatopancreas of pink shrimp and least in muscle and exoskeleton (Nimmo et al., 1971). We found that concentrations of methoxychlor were highest in the exoskeleton, gill, and hepatopancreas, in decreasing order, with less than a two-fold difference in methoxychlor levels between these tissues. The gills contained the highest concentration of methoxychlor calculated on the basis of dry weight, but even so, computed levels were only about three times higher than those in the exoskeleton.

It was calculated that the exoskeleton accounted for some 81% of the methoxychlor found in whole crabs. A large portion, at least 39%, of this value was shown to be associated with the surface of the exoskeleton. The extent to which the pesticide was taken up by the epiflora or adsorbed to the epicuticle is not known. Johnson et al. (1973) reported that uptake of methoxychlor by bacteria was rapid and reached biomagnification levels of 3,400x in 24 hr. Certainly adhering algae and bacteria could account for a large portion of whole-body pesticide levels given in the literature for other crustacea.

Salinity Tolerance of Methoxychlor Exposed Crabs

A preliminary indication of the effect of exposure of adult crabs to methoxychlor on osmoregulatory responses was obtained by studying the relative survival of treated and control crabs exposed to low salinities. There was a clear indication that the crabs treated with pesticide were less resistant to the low salinity exposures (Table 17). Although no deaths

TABLE 17. PERCENTAGE MORTALITY OF ADULT CRABS EXPOSED TO METHOXYCHLOR IN DILUTE SEAWATER

Treatment	Salinity exposure (°/oo)			
	26.5	17.1	9.7	5.5
2 days				
Control	0	0	29	43
Methoxychlor (10 μg/liter)	0	0	47	73
7 days				
Control	17	7	64	100
Methoxychlor (10 μg/liter)	13	7	100	100

occurred at the higher two salinities tested, within two days mortalities were occurring in *C. magister* at 9.7 and 5.5 °/₀₀ salinity. The percentage mortality of crabs exposed to methoxychlor at this time was nearly twice that for the controls. By seven days 100% mortality was noted in both groups of crabs exposed to 5.5 °/₀₀ salinity and also in the methoxychlor treated crabs at 9.7 °/₀₀, but only 64% mortality was noted in the controls at this salinity. Alspach (1972) reported that adult *C. magister* held in 25% seawater were unable to survive for longer than 48 hr, and that crabs exposed to 50% seawater survived for only three days before becoming moribund.

Effects of Methoxychlor on Osmotic and Ionic Regulation

Decreased survival at low salinities could be caused by a reduced osmoregulatory response in the pesticide treated crabs. Kinter et al. (1972) have shown that the osmolarity and the Na^+ and K^+ concentrations in *Anguilla rostrata* serum are significantly elevated following a 6 hr exposure to 1 ppm DDT in seawater, a concentration of the pesticide which is fatal in 10 hr. In additional studies with killifish, *Fundulus heteroclitus*, both DDT and Aroclor 1221 significantly elevated serum osmolarity but only Aroclor 1221 exposure caused elevation of serum Na^+ levels (Kinter et al., 1972). To determine whether similar effects on osmotic and ionic regulation occurred in crabs, we exposed adult *C. magister* to 10 μg/liter methoxychlor in seawater for 14 days and subsequently held the crabs for two more days in a series of reduced salinities. The osmoregulatory response is illustrated in Figure 22. Blood osmotic concentrations of the pesticide exposed crabs did not differ from that of the controls at any of the salinities examined. The results confirm the observations of Alspach (1972) that *C. magister* is a weak hyper-osmoregulator at low seawater salinities. Examination of two of the pesticide group of crabs confirmed that the treated crabs had methoxychlor residues similar to those reported earlier in methoxychlor killed crabs (Table 14), an indication that the pesticide concentration employed in this experiment was only slightly sublethal.

Blood Na^+ regulation was also unaffected by methoxychlor treatment (Figure 23). The pattern of regulation of this ion was essentially the same as that observed for osmotic concentration, consistent with the fact that the Na^+ ion is the major cation found in the blood. The pattern of urine Na^+ concentrations at salinities less than 30 °/₀₀ was comparable to that of the blood in both groups, confirming the extrarenal regulation of this ion at salinities less than full strength seawater (Alspach, 1972; Engelhardt and Dehnel, 1973). In full strength seawater salinities and higher, some regulation of blood Na^+ by reabsorption of this ion from the urine has been demonstrated (Alspach, 1972; Engelhardt and Dehnel, 1973). Our crabs exposed to 30 °/₀₀ salinity had urine Na^+ levels less than that of the blood, confirming this relationship. Although this response was observed in both the methoxychlor exposed and control crabs, the differences between blood and urine Na^+ concentrations were greatest in the control animals.

Blood K^+ regulation (Figure 24) appeared to be poorer at low salinities than that reported by Engelhardt and Dehnel (1973) but similar to the results reported by Alspach (1972). Our results with blood K^+ levels were highly variable and exhibited certain spurious responses. For example, blood K^+ in

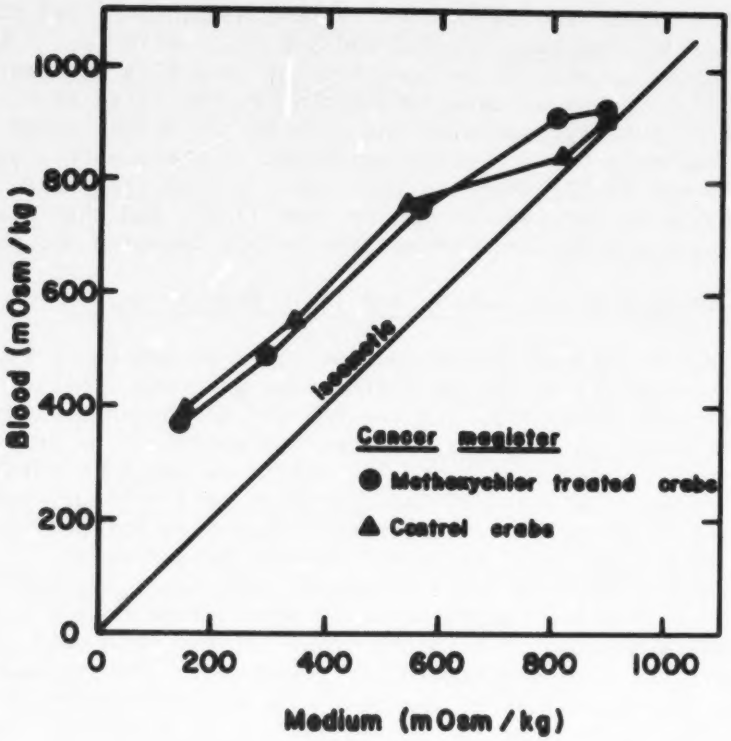


Figure 22. Blood osmotic concentration of control and methoxychlor treated *Cancer magister* as a function of medium osmolarity. Each point represents the mean of analyses on from 7 to 11 crabs.

control crabs was significantly lower ($p<0.05$; Student's t test) at 5 °/oo salinity than that found in methoxychlor-treated crabs. However, this response was reversed at about 28 °/oo salinity where methoxychlor-treated crabs had lower blood K^+ levels than those found in the control animals. At the higher salinities used, the urine to blood ratio was less than unity supporting the contention of renal K^+ regulation in high salinities (Alspach, 1972; Engelhardt and Dehnel, 1973). However, except for the spurious observations already mentioned, blood and urine K^+ levels were not significantly different in comparisons between the control and methoxychlor-treated groups at any of the salinities examined, indicating that the pesticide exposure had no effect on the regulation of this ion.

As previously reported in *C. magister* (Alspach, 1972; Engelhardt and Dehnel, 1973) and in several other crustacean species (Gross, 1964; Gross and Capen, 1966), blood Mg^{++} was strongly regulated to levels below that of the seawater medium (Figure 25). The only indication that methoxychlor treatment affected the blood Mg^{++} concentration was seen at 28 °/oo salinity where the pesticide-treated crabs had significantly higher ($p<0.05$) blood Mg^{++} levels

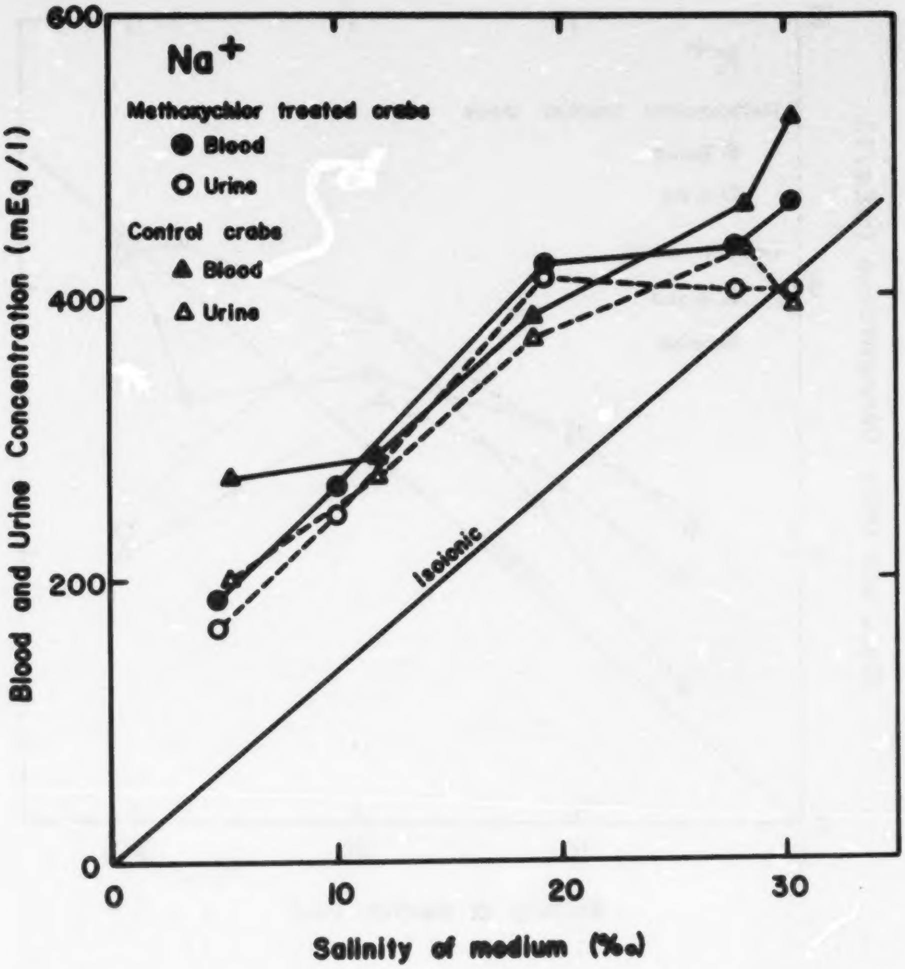


Figure 23. Blood and urine sodium concentration of control and methoxychlor treated Cancer magister as a function of experimental salinity. Each point represents the mean of analyses on from 7 to 11 crabs.

than that seen in the controls. However, this difference was not observed in crabs exposed to 30 °/₀₀ salinity.

Control animals exposed to the higher salinities produced urine in which the Mg^{++} concentrations were appreciably elevated over that of the blood and the medium (Figure 25). However, crabs treated with methoxychlor failed to produce a urine concentrated in Mg^{++}. In view of our inability to demonstrate that methoxychlor affected the regulation of Na^+ and K^+, this observation is of particular interest. The regulation of blood Mg^{++} by crabs has been shown to be a function of the excretory organs, specifically the bladder wall (Gross and Capen, 1966). Although the highest urine Mg^{++} concentrations

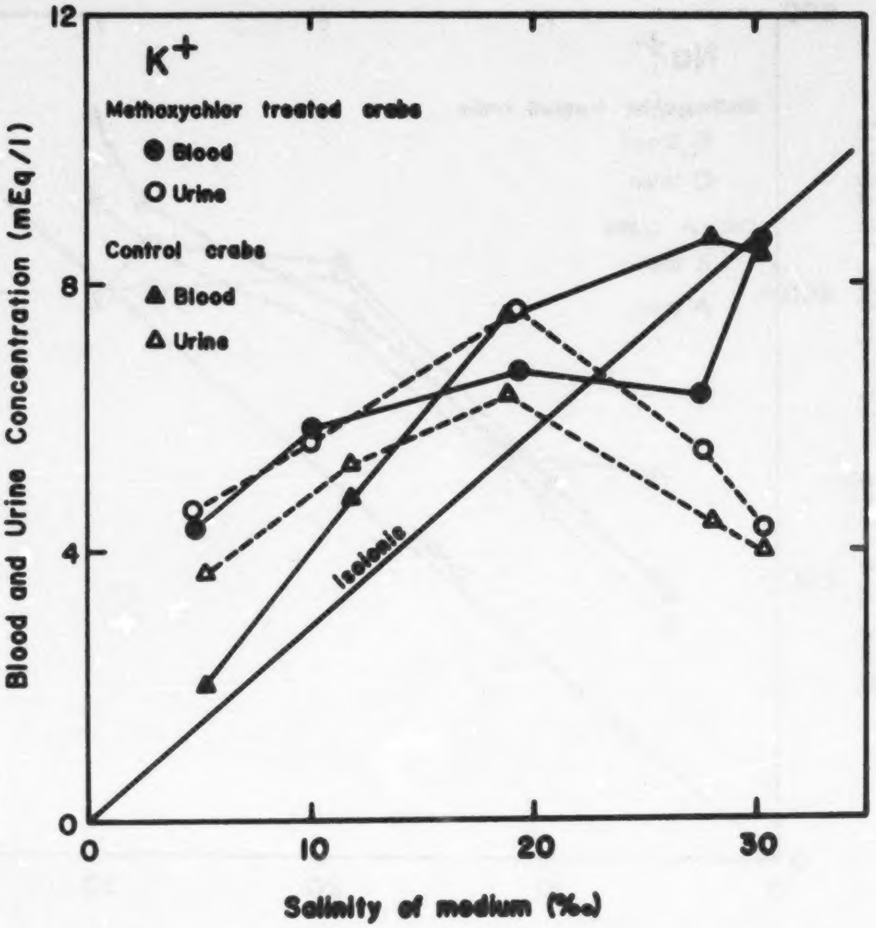


Figure 24. Blood and urine potassium concentration of control and methoxychlor-treated Cancer magister as a function of experimental salinity. Each point represents the mean of analyses on from 7 to 11 crabs.

have been found in crabs immersed in normal and concentrated seawater, Gross and Marshall (1960) demonstrated that, in Pachygrapsus crassipes, the total amount of Mg^{++} excreted per day may be highest when crabs are placed in 50% seawater; since the rate of urine production is appreciably increased in dilute media. The rate of Mg^{++} transport across the bladder wall appears to be a direct function of the blood Mg^{++} level (Gross and Capen, 1966), although this property seems to be better developed in Carcinus maenas than in P. crassipes (Lockwood and Riegel, 1969). Associated with the secretion of Mg^{++}, Na$^+$ is reabsorbed from the urine, presumably by direct exchange in order to achieve electrochemical balance (Gross and Capen, 1966). We have observed that urine Na$^+$ concentration is depressed relative to blood Na$^+$ at a salinity of 30 $^{\circ}/_{\circ\circ}$ (Figure 23). Although the urine Na$^+$ concentration of

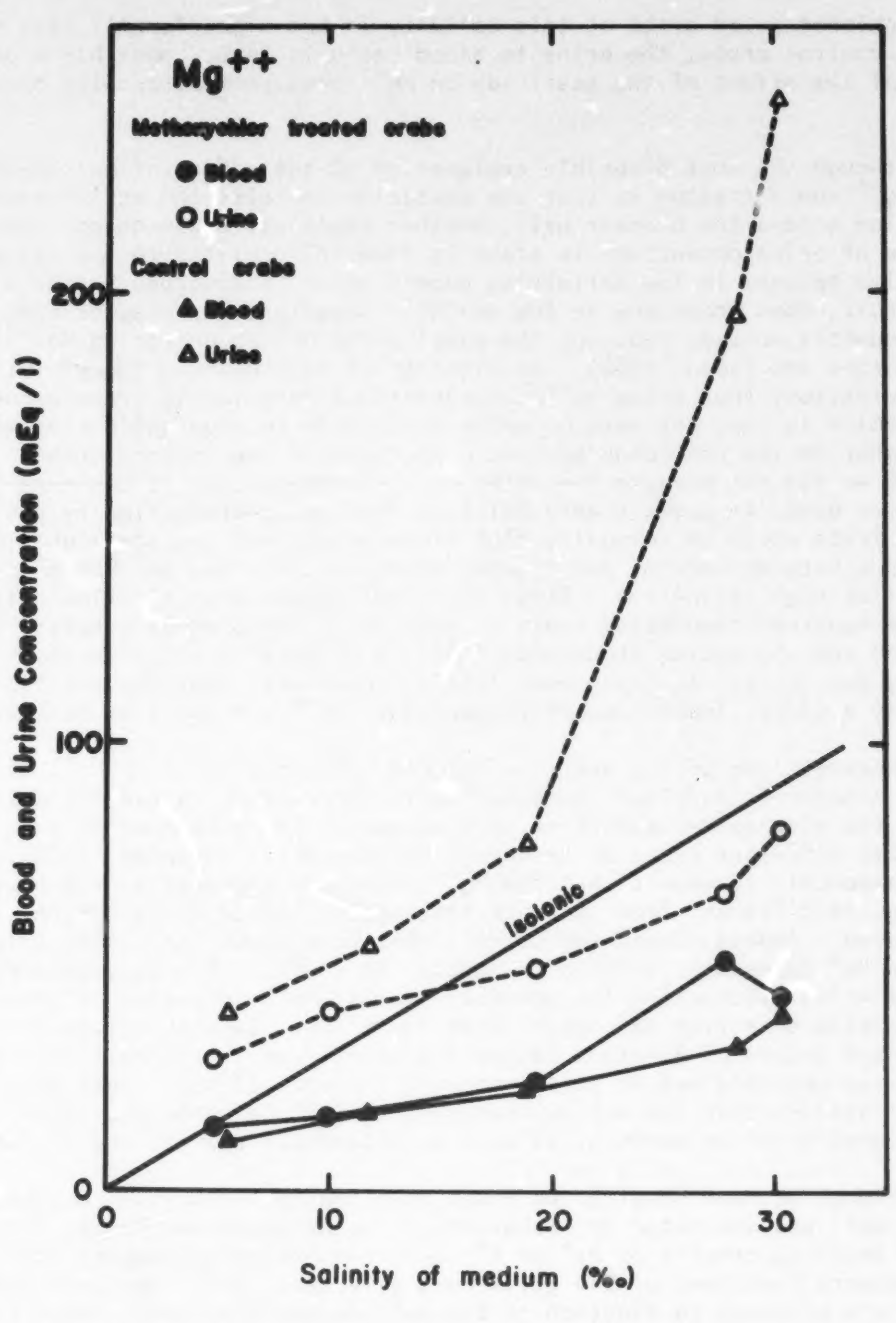


Figure 25. Blood and urine magnesium concentration of control and methoxychlor-treated Cancer magister as a function of experimental salinity. Each point represents the mean of analyses on from 7 to 11 crabs.

methoxychlor-treated crabs at this salinity is not significantly less than for the control crabs, the urine to blood ratio is lower, possibly a consequence of the effect of the pesticide on Mg^{++} transport across the bladder wall.

Although the most plausible explanation of the effect of methoxychlor on urine Mg^{++} concentration is that the pesticide inhibits the active transport of the ion across the bladder wall, another explanation can be considered. The rate of urine production in crabs is inversely related to the salinity of the medium because in low salinities excess water is absorbed by the animal. As a result, when crabs are in low salinity seawater, the bladder contents are frequently voided, reducing the possibility of concentrating Mg^{++} in the urine (Gross and Capen, 1966). An alternative explanation, therefore, for our observations that urine Mg^{++} concentrations were low in crabs exposed to methoxychlor is that the rate of urine production in high salinities was much higher in the pesticide-exposed crabs than in the control crabs. Although we did not measure the rates of urine production at the experimental salinities used, it seems highly unlikely that urine production by pesticide-exposed crabs would be unusually high since we did not see any significant difference between control and treated crabs in the urine Na^+ and K^+ concentrations at high salinities. Also, it is well known that the flow rate of urine in hyperosmoregulating crabs is related to the osmotic gradient between the blood and the medium (Lockwood, 1967), a difference which increases as salinity decreases. It seems most likely, therefore, that our results were caused by a direct inhibition of transbladder Mg^{++} transport by methoxychlor.

Interpretation of the specific effects of methoxychlor on Mg^{++} transport in crab bladder is difficult because little information is available concerning the biochemical nature of this process. There is considerable evidence that different types of transport enzymes exist (Oxender, 1972) so that it is reasonable to expect that the Mg^{++} transport process in crab bladder may be quite different from the more thoroughly studied NaKMg ATPase transport system. Indeed, Gross and Capen (1966) have shown that crabs treated with the Na^+ transport inhibitor, ouabain, were still able to transport Mg^{++} into the urine,discounting the possibility that Mg^{++} secretion is enzymatically coupled to active Na^+ uptake from the urine. Similar evidence for the independent nature of divalent cation transport from the NaKMg ATPase transport system was obtained by Schatzmann and Vincenzi (1969). They have shown in their studies that the active transport of Ca^{++} in human red blood cells is also unaffected by ouabain, as well as oligomycin and Na^+ and K^+ ions.

Although we have obtained evidence that the Mg^{++} transport system in the bladder wall was inhibited by methoxychlor, no evidence was found, in the form of impaired osmotic or Na^+ or K^+ ionic regulation to suggest that the ion transport functions of the gills were affected. Since the gill NaKMg ATPases are presumed to function in the Na^+ transport process, these results suggested that the NaKMg ATPases in crab gills may not have been inhibited by the pesticide. Davis et al. (1972) have shown that the ATPase activities of rainbow trout gill are inhibited by methoxychlor in vitro, but the amount of inhibition produced was considerably less than that found with a number of other organochlorine insecticides. To determine the effect of methoxychlor on crab gill ATPases, gills were excised from adult C. magister which had been held in 10 µg/liter methoxychlor for a 14-day period. The control group was treated in an identical fashion, but was exposed only to acetone. The gill tissue from two animals was pooled and activity was measured in whole homogenates (Table 18). NaKMg ATPase activity was significantly ($P<0.05$) inhibited by methoxychlor treatment to the extent of 37%. Inhibition of both the Mg and NaK components of the enzyme appeared to be similar, 36 and 50%, respectively, but the differences were not significant.

TABLE 18. ATPASE ACTIVITY IN GILLS OF ADULT CRABS EXPOSED TO METHOXYCHLOR

Treatment	ATPase activity (µmoles P_i/mg protein/hr)*		
	NaKMg	Mg	NaK
Control	2.13 ± 0.32 (4)	1.83 ± 0.60 (4)	0.34 ± 0.35 (4)
Methoxychlor (10 µg/liter)	1.34 ± 0.34 (5)	1.17 ± 0.37 (5)	0.17 ± 0.25 (5)

* Data are the mean ± two standard errors of the mean. The numbers in parentheses indicate the number of individual analyses conducted per treatment.

The reasons for the apparent contradiction between the effect of methoxychlor on gill ATPase activity and the lack of effect of the pesticide on blood osmotic regulation and on regulation of blood Na^+ and K^+ in the crab are difficult to understand. The activity of the Na^+ transport system in crustacean gills is influenced by both the blood and medium concentrations of this ion (Lockwood, 1962). It is possible that the degree of inhibition of the enzyme was insufficient to significantly affect its function in osmotic and ionic regulation of the blood if the requirement for activity was less than the maximum capacity of the enzyme in normal crabs. This is plausible since the capacity for enzyme catalyzed reactions often greatly exceeds the rates required in vivo (Chance and Hess, 1959). The correlation between gill ATPase inhibition and gross toxicological effects is equivocal in other studies as well. Davis et al. (1972) in comparing literature values of pesticide chemicals, reported that the chlorinated hydrocrabon insecticides were more lethal and also more effective inhibitors of gill ATPases than the herbicides, whereas PCB's were intermediate in effect. However, the same direct relationship was not found within the insecticide group alone, which suggests that the inhibition of gill ATPase is coincidental to a primary lethal lesion. If the primary lesion is associated with ion distributions across axonal membranes, as has been previously suggested (O'Brien, 1967), possibly due to an effect on the membrane NaKMg ATPases of the axon, then a partial but imperfect correlation with gill ATPases is not surprising. Davis et al. (1972) also reported that trout given lethal doses of DDT orally accumulated high tissue residue levels in gills, brain and kidney; but that no differences in ATPase activities of these tissues were observed between treated and control groups of fish, lending further support to this supposition.

Our initial assumption in this study was that methoxychlor, as is the case with other organochlorine pesticides and PCB's examined (Davis et al., 1972), would inhibit the NaKMg ATPase activity of gills and consequently impair osmotic and ionic regulation in the crabs, an effect which should ultimately reduce the tolerance of the animals to low salinity exposures. Our results with gill ATPase in C. magister confirmed that the pesticide administered to crabs at barely sublethal levels resulted in inhibition of the enzyme (Table 18). However, with the exception of Mg^{++} regulation, we have been unable to show any adverse effect on osmotic and ionic regulation. Yet, despite this apparent lack of effect on osmotic and ionic regulation, the crabs, after exposure to the pesticide, exhibited a decreased tolerance to low salinity exposures (Table 17). Although the role of Mg^{++} regulation is uncertain, these results suggest that impairment of osmotic and ionic regulation in crabs by organochlorine pesticides is not the principle effect leading to decreased survival at low salinities.

Exposure to low salinities, even in normal crabs, is accompanied by a reduction in blood osmotic and ionic concentrations (Alspach, 1972; Dehnel, 1962; Dehnel and Carefoot, 1965; Engelhardt and Dehnel, 1973). It is conceivable, therefore, that the normal reduction of osmotic and ionic content of the blood may be potentiating the known physiological effects of organochlorine pesticides on the nervous system. The familiar symptoms of poisoning by this class of compounds, which were also seen in the present study in methoxychlor-poisoned crabs, begins with the hyperactive state and at high dosages is eventually followed by loss of motion and paralysis. Individual nerve axons of diverse species poisoned by DDT are characterized by uncontrolled volleys of impulses (Yeager and Munson, 1945). It is postulated that the DDT-like organochlorines exert their effects on nerves by blocking the membrane Na^+ and K^+ channels and by inhibiting the nerve ATPases (Matsumura and Narahashi, 1971; Matsumura and Patil, 1969; Narahashi and Hass, 1967). It is reasonable to suppose that normal nerve function occurs within a finite range of blood ionic concentrations with optimum conditions near the center of that range. We postulate that damage to nerve axons, resulting from sublethal exposure to methoxychlor, serves to reduce the range of blood ionic concentrations compatible with continued nerve function with the result that survival of crabs at extreme salinities is reduced.

THE HERBICIDES 2,4-D, DEF, PROPANIL AND TRIFLURALIN

Exposure of eggs to trifluralin, DEF and propanil accelerated hatching compared with controls at all concentrations tested (Figures 26-28). The hatching success during 24 hr for each of these compounds was in the range of 55 to 70% while that for controls was only 39%. The response to 2,4-D exposure differed from that of the other herbicides (Figure 29). At the two lowest test concentrations, 3,300 and 10,000 μg/liter, eggs hatched with a success of 80% or higher. At 33,000 and 100,000 μg/liter 2,4-D, hatching success was comparable to that found with the other herbicides, 60 to 70%, but at the highest concentration tested, 330,000 μg/liter, the hatching of eggs was almost completely inhibited; only one egg out of 58 hatched. At the latter concentration all of the unhatched eggs appeared larger and were more opaque than were unhatched control eggs.

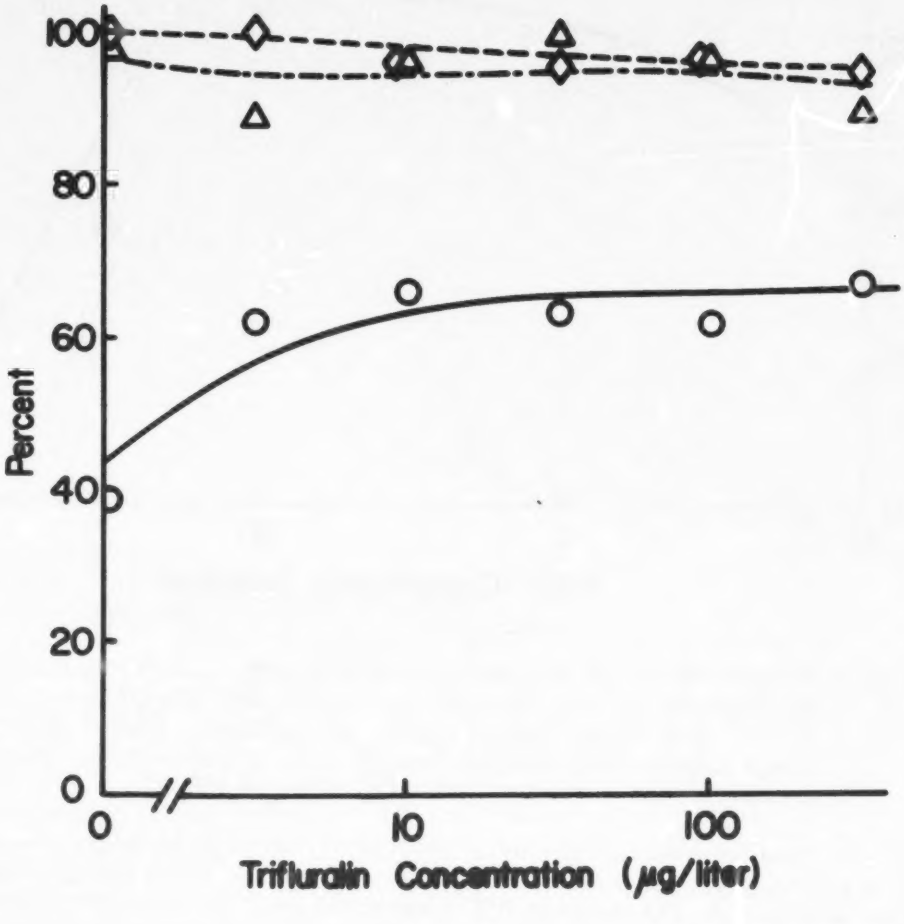


Figure 26. Percent hatch of Dungeness crab eggs (○——○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—·—△), and percent of developed first stage zoeae which are motile (◇-- --◇) as a function of trifluralin concentration during a 24-hr exposure period.

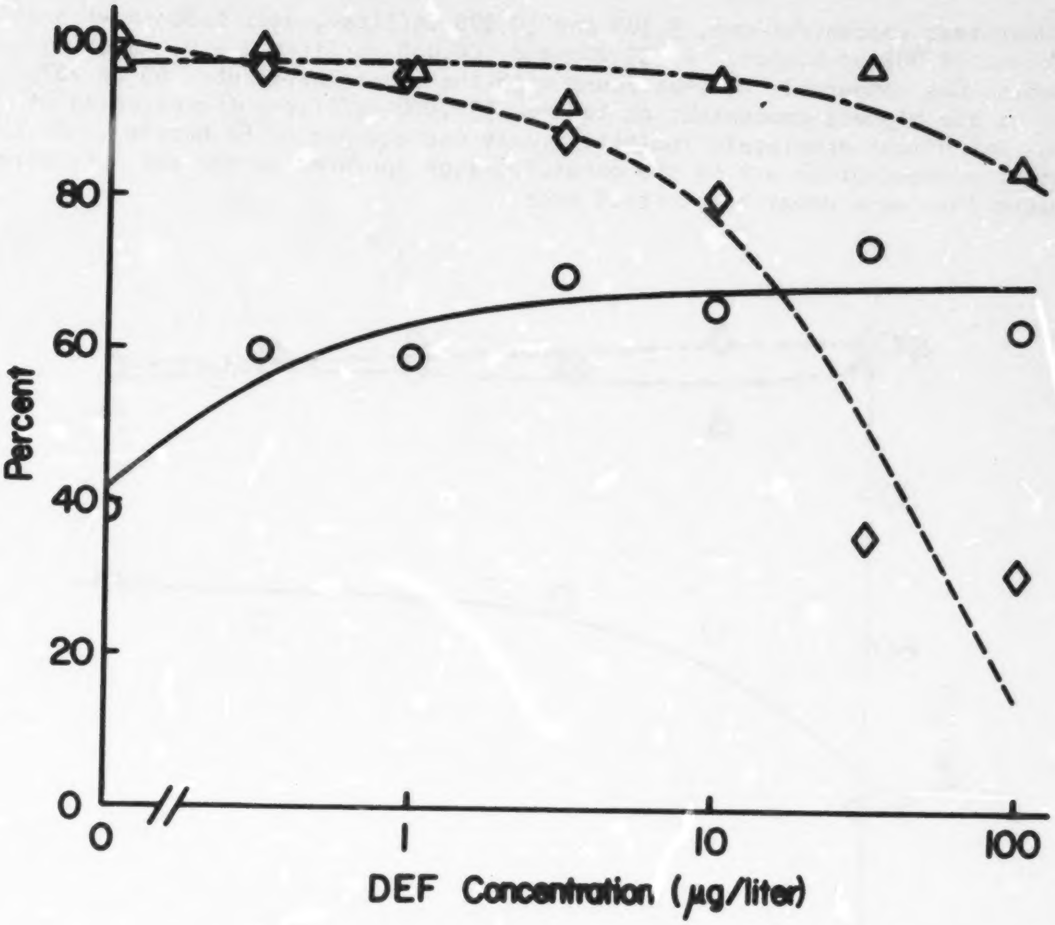


Figure 27. Percent hatch of Dungeness crab eggs (○——○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—·—△), and percent of developed first stage zoeae which are motile (◇----◇) as a function of DEF concentration during a 24-hr exposure period.

At all test concentrations of trifluralin, DEF and propanil, more than 80% of hatching prezoeae successfully molted into the first zoeal stage (Figures 26-28). At the highest DEF concentration, 100 µg/liter, there was an indication that the success of this development was being affected. As was the case for egg hatching, the effect of 2,4-D treatment was more pronounced. No effect was observed at 33,000 µg/liter, but at 100,000 µg/liter less than 20% of the hatching prezoeae successfully developed into first stage zoeae (Figure 29).

Another criterion of the effect of these compounds on early developmental stages was the motility of developing first stage zoeae. No effect

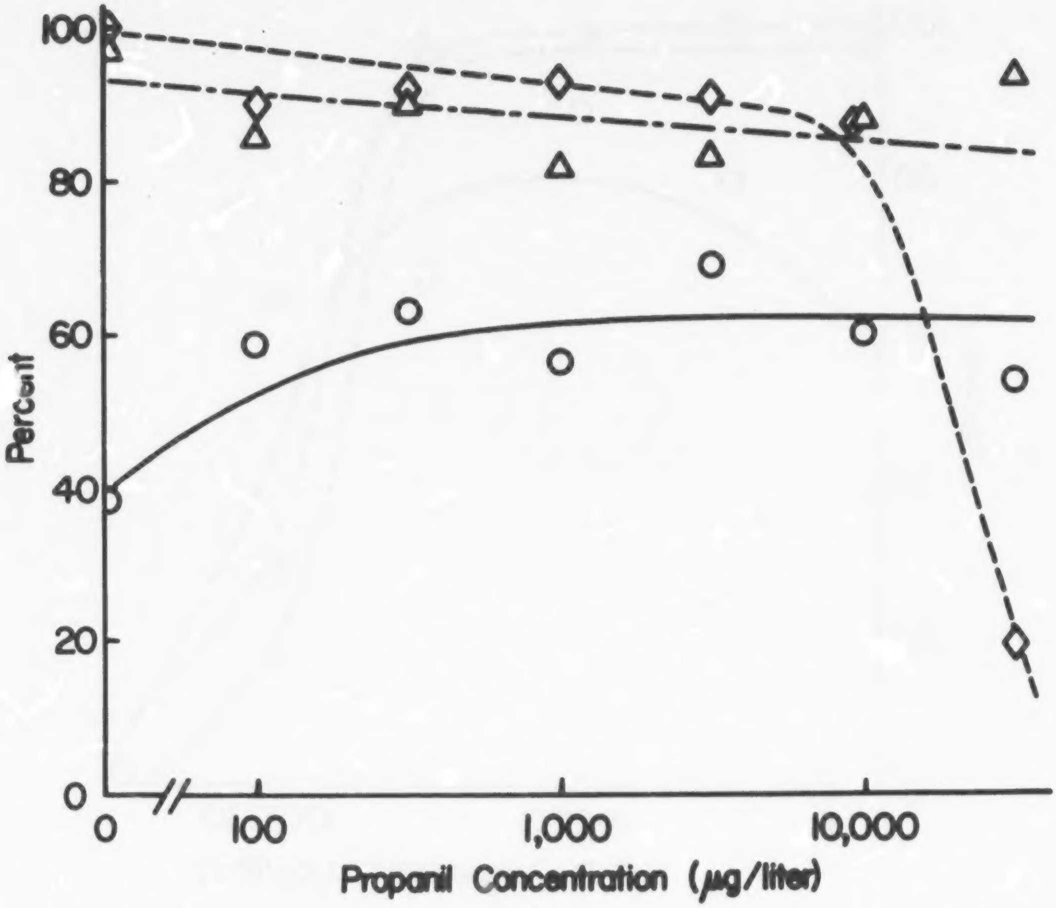


Figure 28. Percent hatch of Dungeness crab eggs (○——○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—·—△), and percent of developed first stage zoeae which are motile (◇----◇) as a function of propanil concentration during a 24-hr exposure period.

on zoeal motility was observed with trifluralin exposure (Figure 26), but effects were noted with both DEF and propanil (Figures 27 and 28). The percentage of zoeae motile after exposure to DEF gradually declined with increasing concentration up to 10 μg/liter at which point only 80% of the zoeae were motile. Motility appeared to be generally unaffected with propanil exposures up to 10,000 μg/liter, but at 33,000 μg/liter only 20% of first stage zoeae were motile. Exposure to 2,4-D did not affect motility of developing first-stage zoeae at concentrations up to and including 33,000 μg/liter. However, most of the few larvae that metamorphosed into first stage zoeae at 100,000 μg/liter were also non-motile.

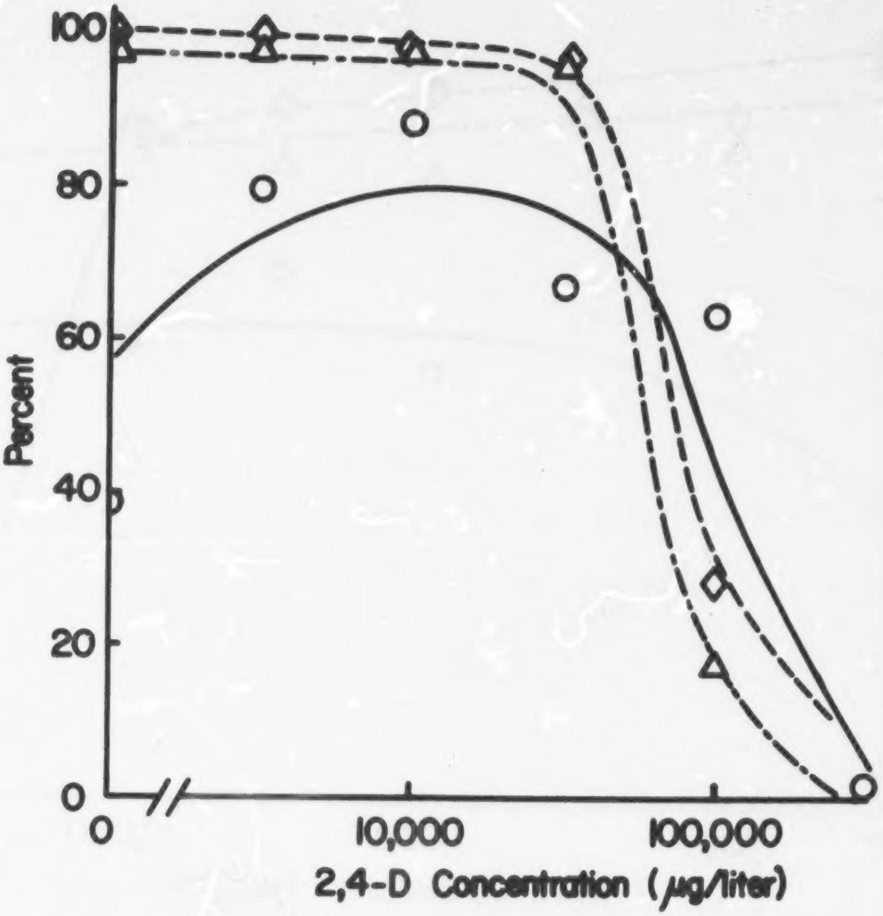


Figure 29. Percent hatch of Dungeness crab eggs (○——○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—·—△), and percent of developed first stage zoeae which are motile (◇----◇) as a function of 2,4-D concentration during a 24-hr exposure period.

The results of 96-hr acute toxicity tests for each of the herbicides tested against first instar zoeae, first instar juvenile crabs and adult crabs are summarized in Table 19. The sensitivity of first stage zoeae to the four herbicides increased in the series 2,4-D, propanil, trifluralin and DEF. No acute lethal effects were seen with 2,4-D at a concentration of 10,000 µg/liter, the highest concentration tested against zoeae. Concentrations of propanil lethal to 50% of the test organisms in 96-hr were 7,300 µg/liter and 1,500 µg/liter, respectively, for the technical and the Stam F-34 formulation. The 96-hr LC50 for technical trifluralin against first instar zoeae exceeded 110 µg/liter, but with the Treflan EC formulation the 96-hr LC50 for trifluralin was 250 µg/liter. Only 6.6 µg/liter of technical DEF was lethal to 50% of the test organisms in 96 hr. For zoeae the same

TABLE 19. ACUTE TOXICITY OF HERBICIDES TO FIRST INSTAR ZOEAE, FIRST INSTAR JUVENILE CRABS AND ADULT CRABS IN 96-HR TESTS. DATA ARE EXPRESSED IN μg/LITER

Pesticide	Zoeae		Juvenile		Adult	
	EC50	LC50	EC50	LC50	EC50	LC50
Trifluralin						
Technical	60	>110	>1,000	>1,000	-	>9,300
Treflan E.C.	140	250	-	-	-	-
DEF						
Technical	1	6.6	170	190	-	>1,000
Propanil						
Technical	2,200	7,300	-	-	-	>26,000
Stam F-34	560	1,500	5,600	6,000	-	-
2,4-D acid						
Technical	>10,000	>10,000	>100,000	>100,000	-	-

relative order of toxicity was noted with these four herbicides as judged by the EC50 criteria, absence of swimming, except that the EC50s were about 18 to 50% of the respective LC50 values.

Juvenile and adult crabs were considerably less sensitive to the herbicides in acute tests than were first instar zoeae (Table 19). In most of these tests with older stages no acute effects were seen even at the highest concentrations tested. Juvenile crabs were 170 and 29 times less sensitive to technical DEF than were first instar zoeae judging from the 96-hr EC50 and LC50s, respectively. Juvenile crabs were 10 times and 4 times less sensitive to propanil (as stam F-34) than were first instar zoeae as judged by the same tests.

Two chronic toxicity experiments were conducted with zoeae and each of the four herbicides. During the first experiment widespread infection of the cultures with the fungus Lagenidium, coinciding roughly with the molt to second instar between days 10 and 15, led to early termination of the experiment on day 18. However, even in this short test, the highest trifluralin and propanil concentrations, 150 and 800 μg/liter, respectively, clearly reduced the survival of the first instar larvae (Figures 30 and 31). No effects of either of these compounds were noted at lower concentrations. Neither 2 μg/liter of DEF nor 10,000 μg/liter of 2,4-D, the highest test concentrations, respectively, had an effect on survival of larvae during this test

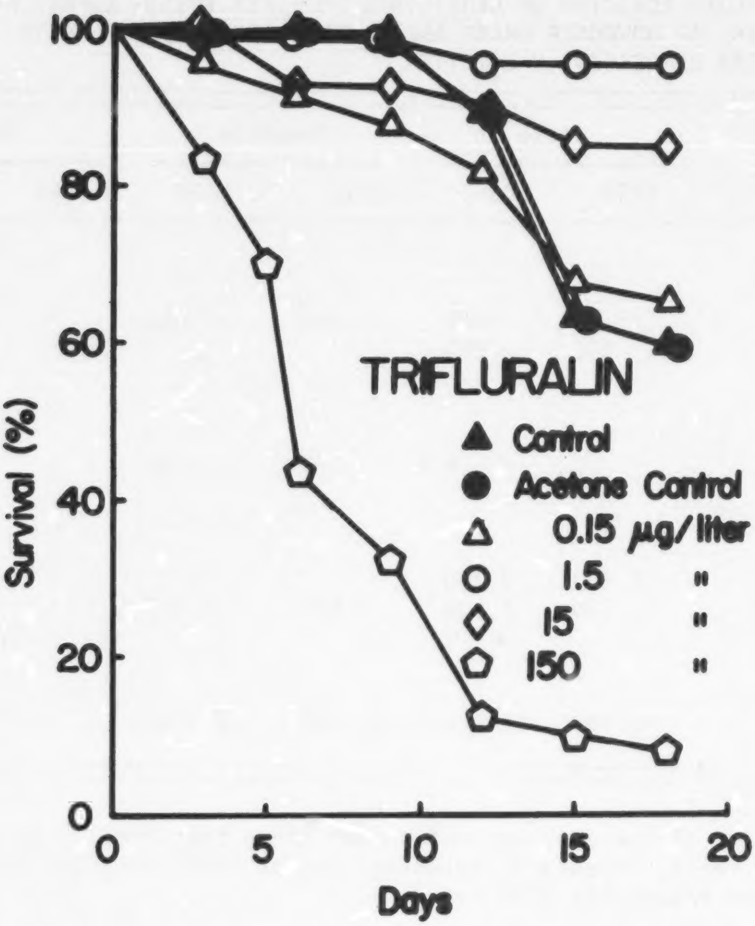


Figure 30. Effect of continuous exposure to trifluralin on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973.

series (Figures 32 and 33).

During the second experiment survival of zoeae in control cultures and in cultures exposed to low concentrations of the herbicides generally exceeded 85% until day 50 (Figures 34-37). Beyond this time increased mortalities occurred in all of the cultures including the controls until the tests were terminated on day 69. Survival in control cultures averaged about 40% at this time. In good agreement with the results of the first experiment, larvae exposed to 150 µg/liter of trifluralin were rapidly killed; no larvae survived beyond day 8 (Figure 34). Similarly, larvae exposed to 800 µg/liter of propanil were rapidly killed as in the first experiment. Survival at this concentration was only 10% on day 20, but some of the remaining larvae survived an additional 35 days (Figure 35). As in the first test, larvae

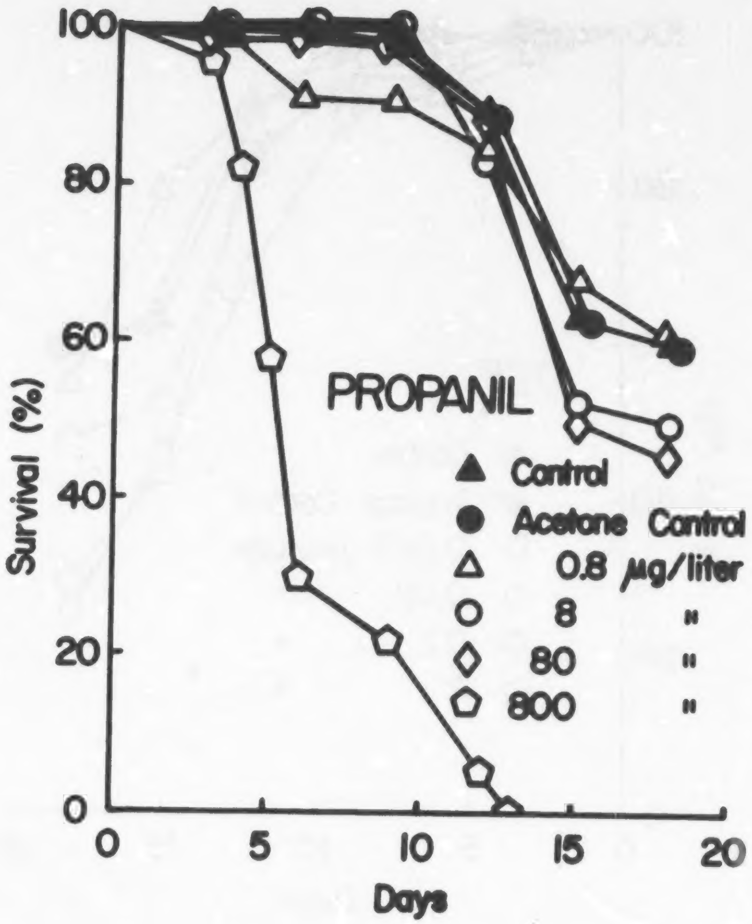


Figure 31. Effect of continuous exposure to propanil on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973.

exposed to lower concentrations of these two herbicides were entirely unaffected during the entire experiment. Larval survival was also clearly affected by exposure to 10,000 µg/liter of 2,4-D in this experiment (Figure 36). Approximately 50% of the zoeae exposed to this concentration survived to day 20, but less than 10% survived 50 days of exposure. Survival of larvae exposed to 1,000 µg/liter 2,4-D was essentially the same as that in controls. At the highest DEF concentration, 4 µg/liter, no effects were noted on the survival of zoeae (Figure 37).

During the second chronic toxicity experiment we attempted also to determine whether molting was affected in crabs not killed by herbicide exposure. First stage zoeae exposed to 150 µg/liter trifluralin had all died

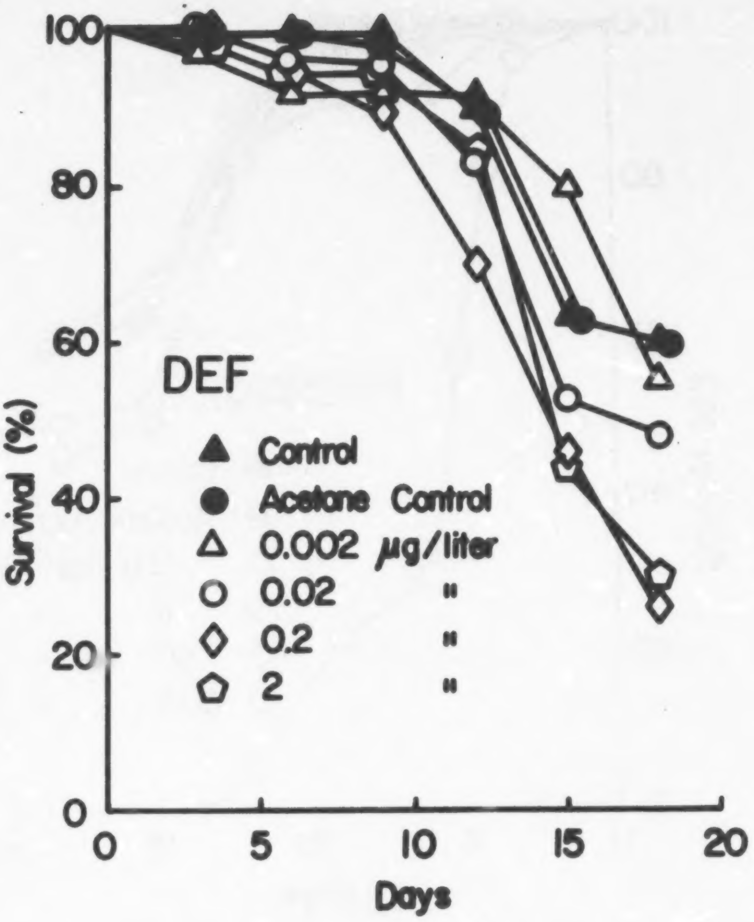


Figure 32. Effect of continuous exposure to DEF on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973.

by day 8 (Figure 34), four days before the controls had molted to the second zoeal stage (Figure 38). However, zoeae exposed to 15 µg/liter, which survived as well as controls, also seemed to molt in synchrony with the control zoeae (Figure 38) indicating that this herbicide does not affect molting. For zoeae exposed to the highest DEF concentration, 4 µg/liter, the first and second molts each occurred about 1 day behind those of control larvae (Figure 39), but by the time of the fourth molt larvae at this concentration were molting as soon or sooner than the controls. These results suggest that DEF also does not affect molting. At sublethal concentrations of propanil, 80 and 8 µg/liter, molting occurred in synchrony with controls, but larvae exposed to a lethal concentration, 800 µg/liter, either failed to molt or molted much later than controls (Figure 40). At this latter concentration, only three first stage zoeae molted, two on day 24 and one on day 32, and

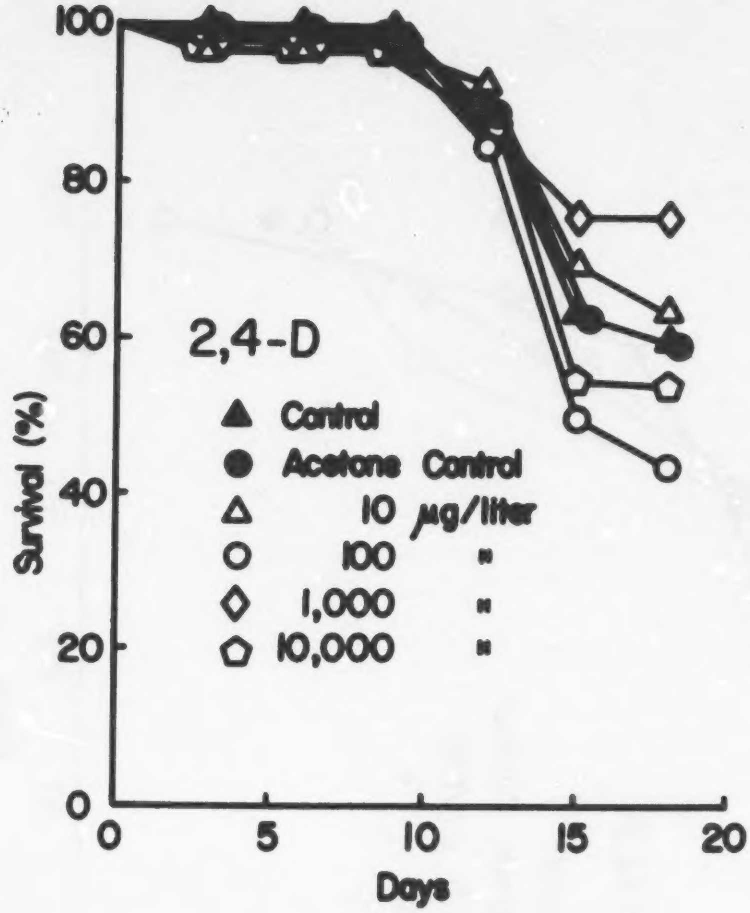


Figure 33. Effect of continuous exposure to 2,4-D on survival of Dungeness crab zoeae. Data are from the first zoeal chronic experiment conducted during the spring of 1973.

none of these molted to the third instar even though one larva survived until day 56.

Exposures to 2,4-D at both 1,000 and 10,000 µg/liter clearly resulted in some delay of molting during the larval development period (Figure 41). Larvae exposed to 1,000 µg/liter were about two to three days delayed in the time to the first molt compared to controls, and this interval was maintained for each of the following three molts. An even greater delay was observed in the first molt of larvae exposed to 10,000 µg/liter. An initial molt was not noted until day 15 at which time essentially all of the control animals had completed the first molt, and of those larvae eventually completing this molt, the median time was about 18 to 19 days, a delay of 5 to 6 days from that of the controls. Because of continued mortalities of the larvae exposed

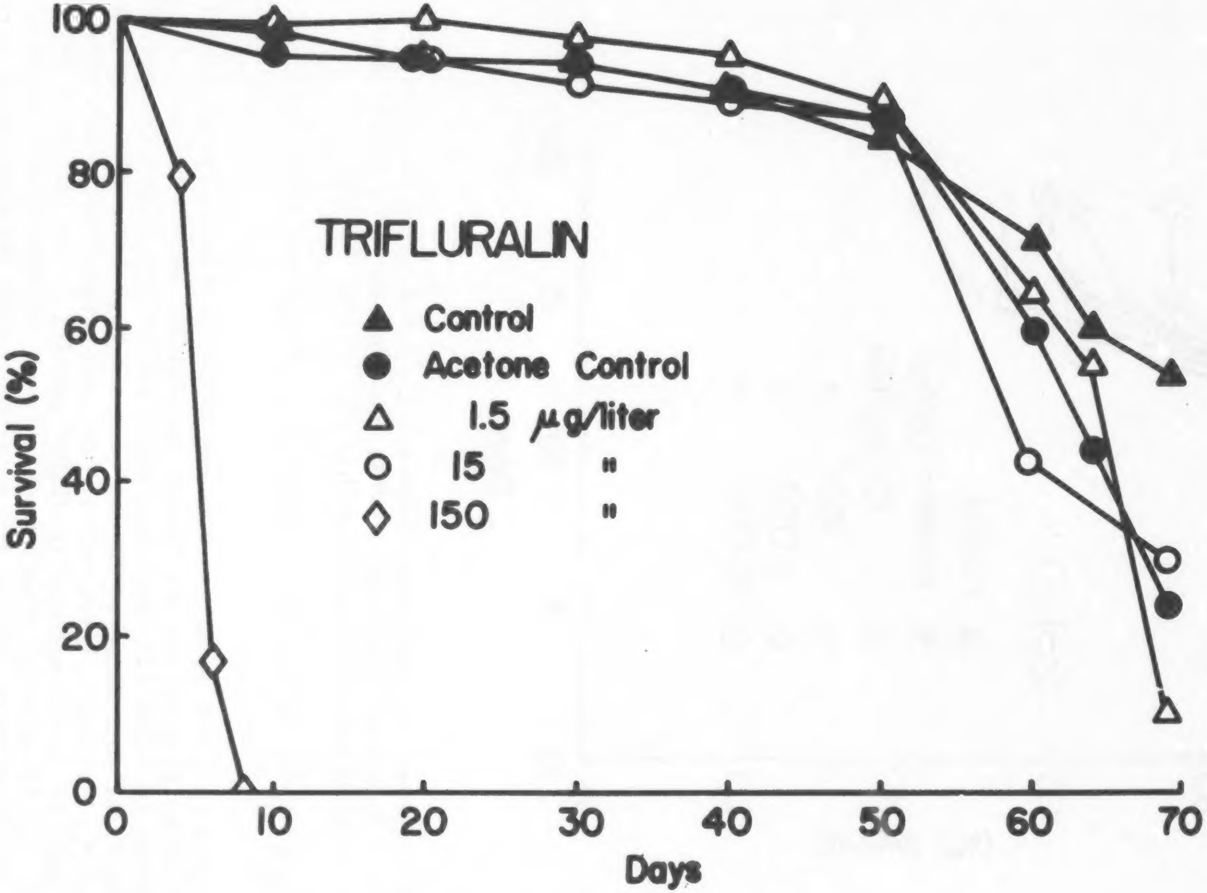


Figure 34. Effect of continuous exposure to trifluralin on survival of Dungeness crab zoeae. Data are from the second zoeal experiment conducted during the spring of 1974.

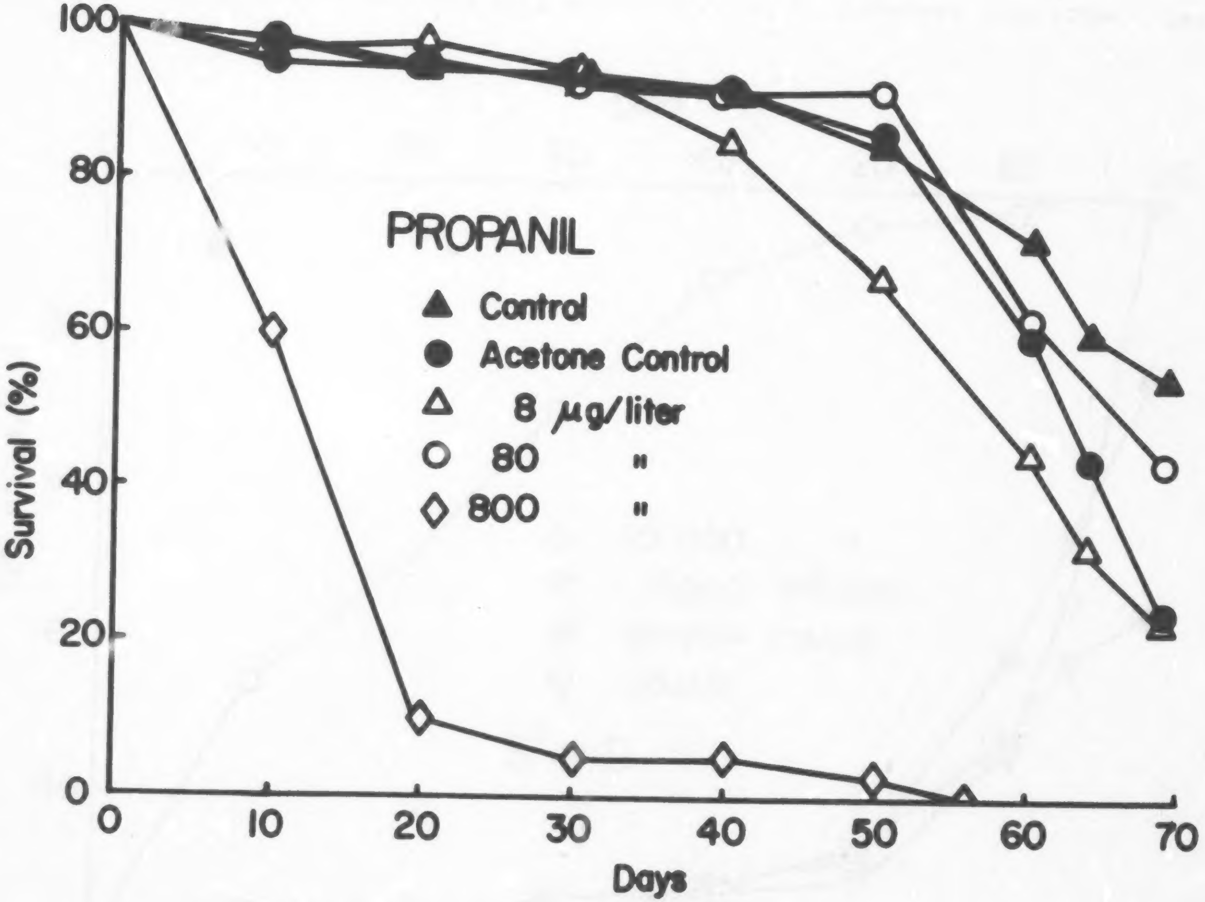


Figure 35. Effect of continuous exposure to propanil on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

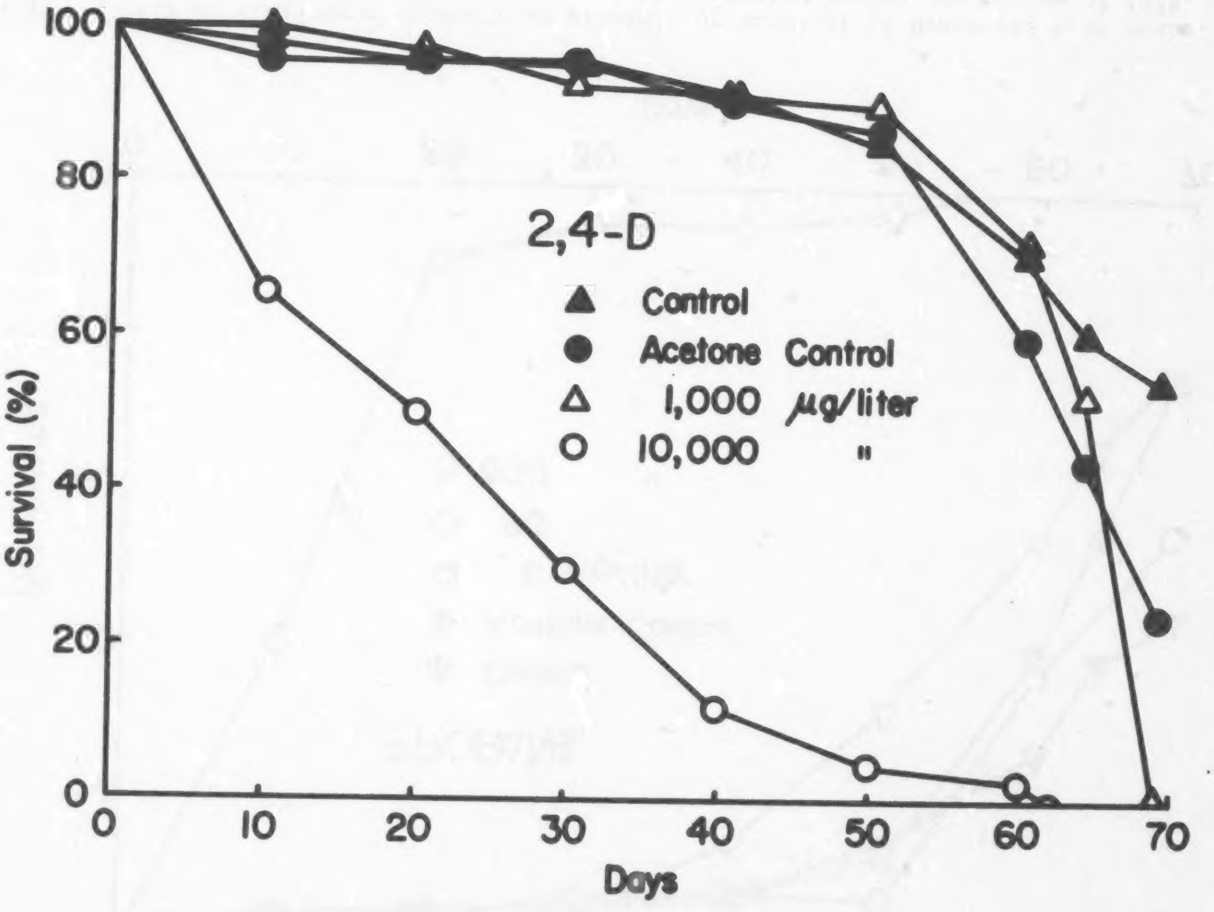


Figure 36. Effect of continuous exposure to 2,4-D on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

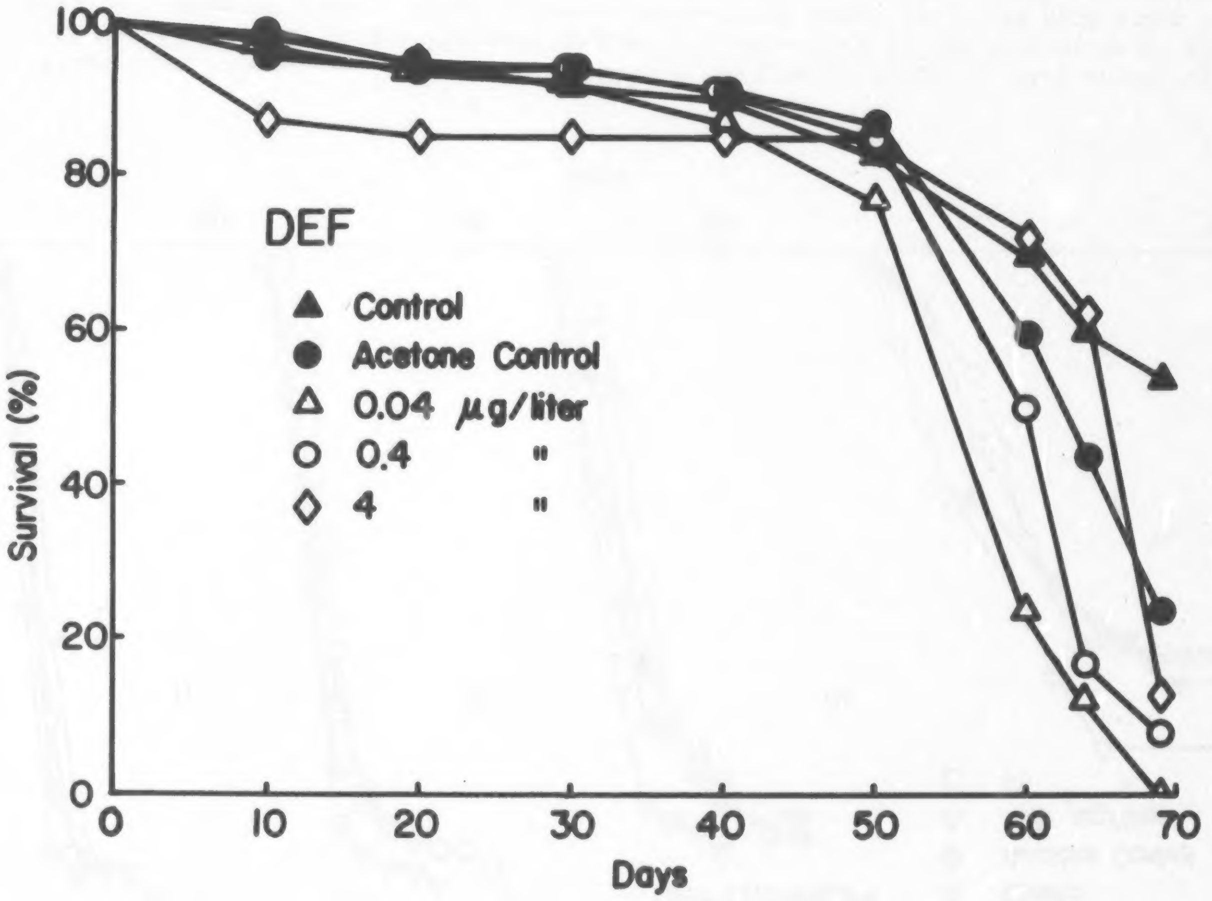


Figure 37. Effect of continuous exposure to DEF on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

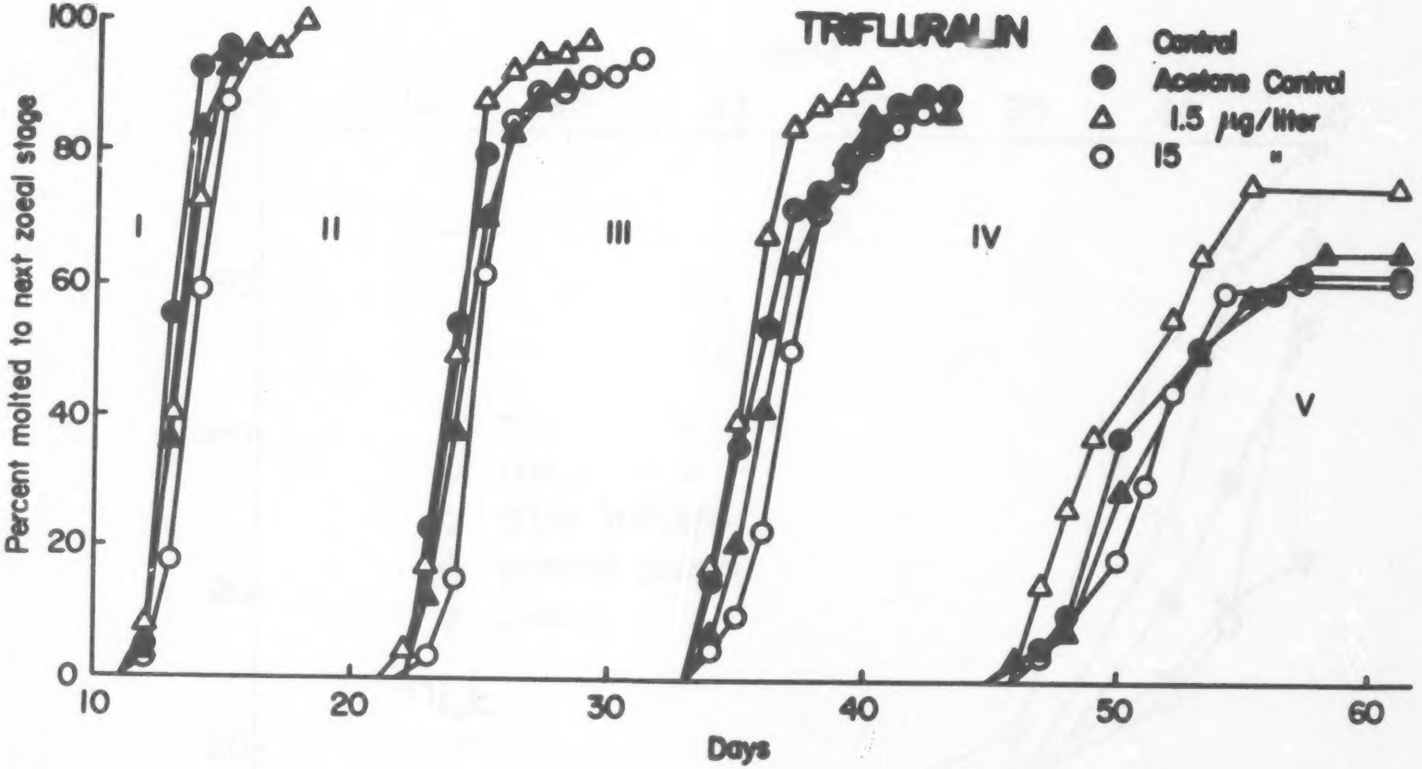


Figure 38. Effect of continuous exposure to trifluralin on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success for each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

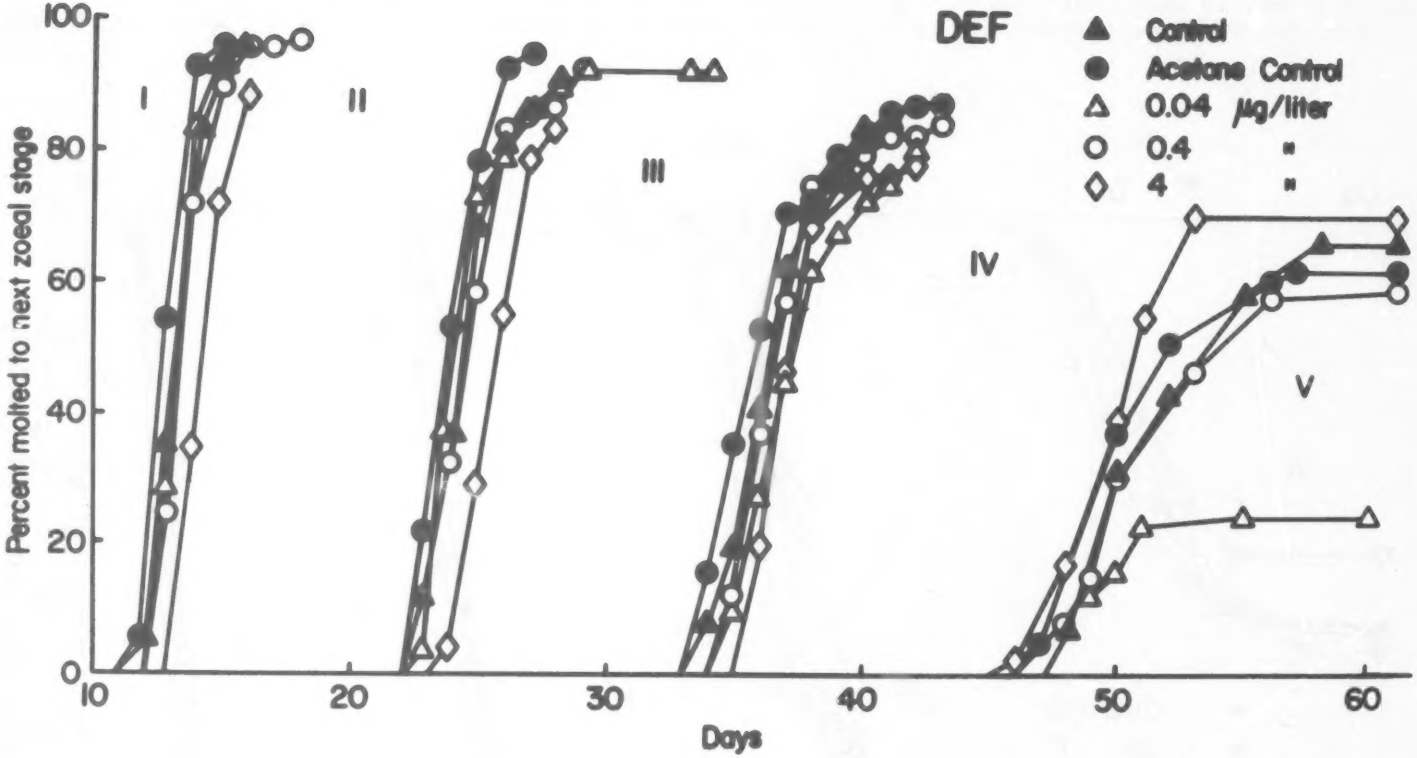


Figure 39. Effect of continuous exposure to DEF on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success for each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

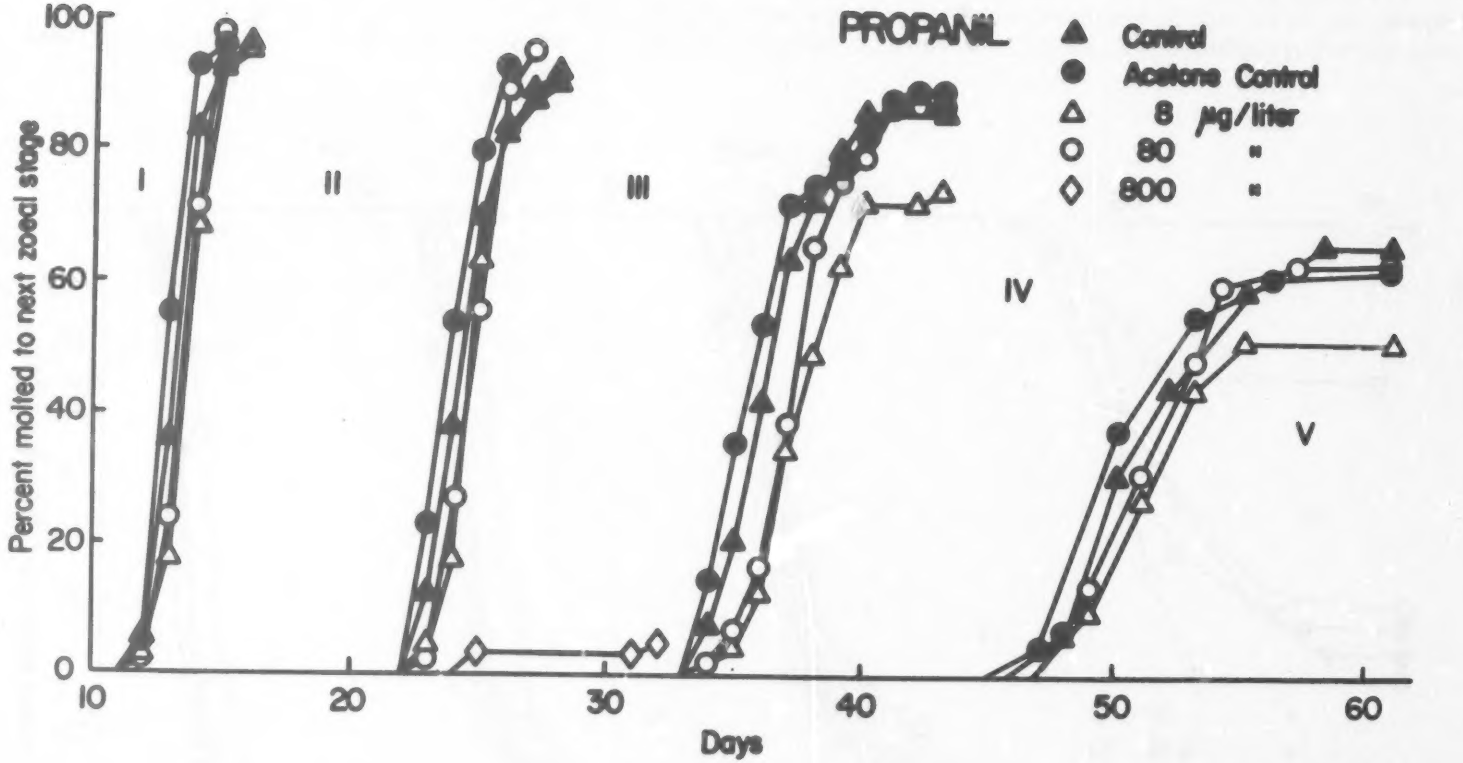
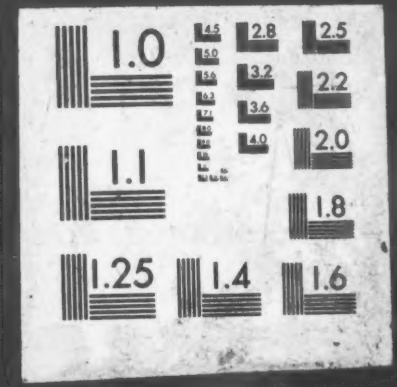


Figure 40. Effect of continuous exposure to propanil on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success for each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

2 OF 2

PB

276 978


1.0
4.5
5.0
5.6
6.3
7.1
8.0
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.1
1.8
1.25
1.4
1.6

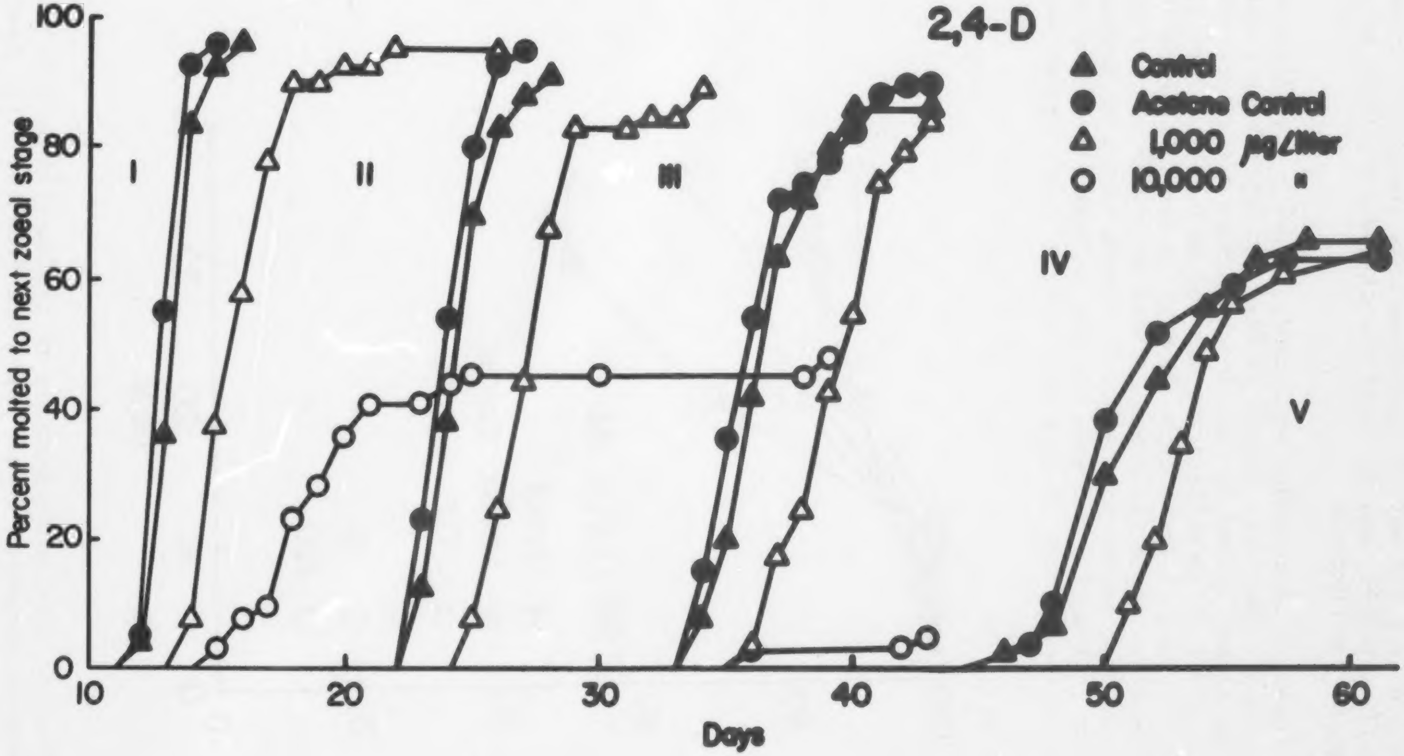


Figure 41. Effect of continuous exposure to 2,4-D on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success for each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

to this concentration of 2,4-D only a few larvae were successful in completing the second zoeal molt, and at least one of these occurred as long as 20 days after that of the controls.

Two chronic toxicity experiments were conducted with juvenile crabs for each of the herbicides: The first lasted 36 days and began with first instar juvenile crabs; the second lasted 80 days in which most crabs were initially third instars. Survival of juvenile crabs exposed to 0.15 μg/liter trifluralin was less than for control crabs after 20 days in the first experiment, but since this effect was absent in crabs exposed to higher concentrations of trifluralin, the response cannot be attributed to the herbicide exposure (Figure 42). Also trifluralin exposure did not influence the time of occurrence of the first juvenile molt (Figure 43). In the second experiment with

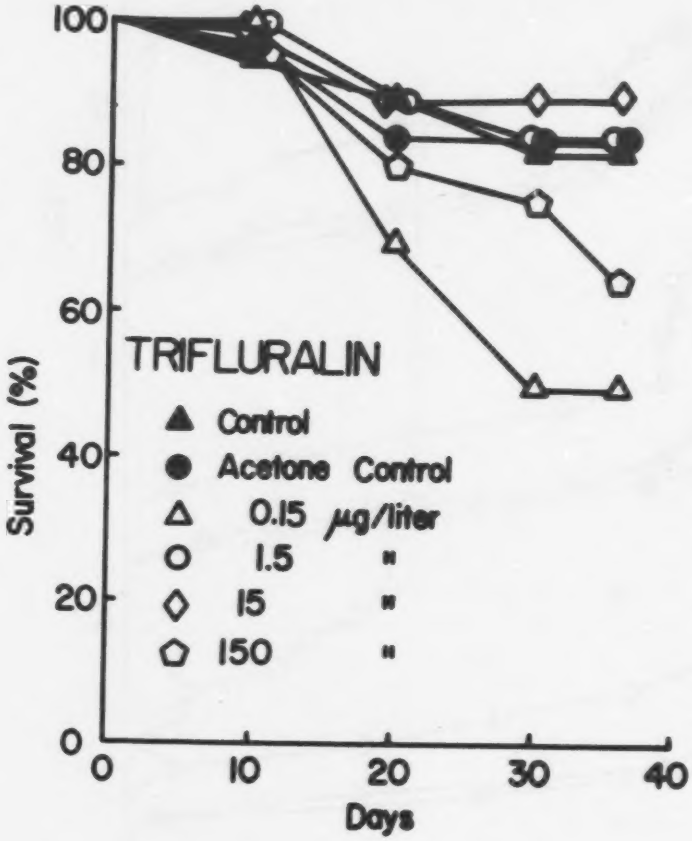


Figure 42. Effect of continuous exposure to trifluralin on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs.

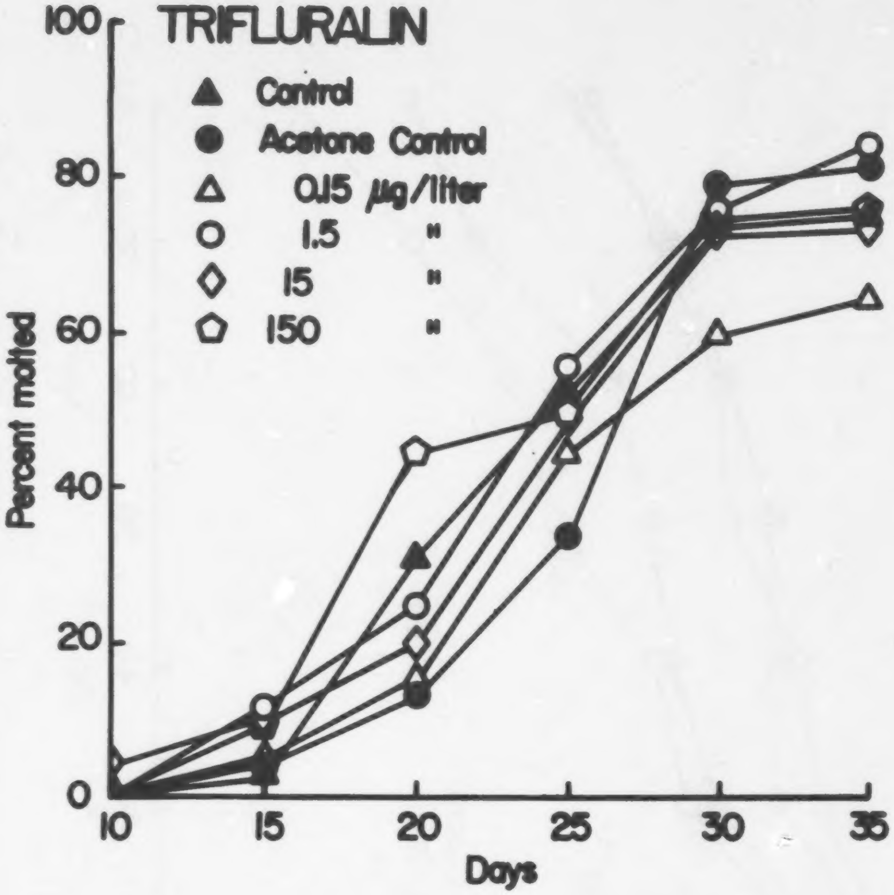


Figure 43. Effect of continuous exposure to trifluralin on molting of first instar Dungeness crabs to the second instar. The molt success is expressed as the percentage of the original number of first instar crabs.

trifluralin, crabs exposed continuously to 15 and 150 µg/liter of the herbicide (the second highest and highest concentrations tested) survived as well as controls (Figure 44). Juvenile crabs exposed to 150 µg/liter of DEF exhibited poorer survival than controls in both the first and second experiments (Figures 45 and 46) but were not affected by exposure to 15 µg/liter. Molting of crabs in the first experiment with DEF was not affected at any concentration; of the crabs surviving to day 35 in each treatment, approximately half had molted by day 24 (Figure 47).

The highest concentration of propanil, 800 µg/liter, did not affect survival of crabs in the first experiment (Figure 48) but was associated with a reduced survival of crabs in the second experiment after day 20 (Figure 49).

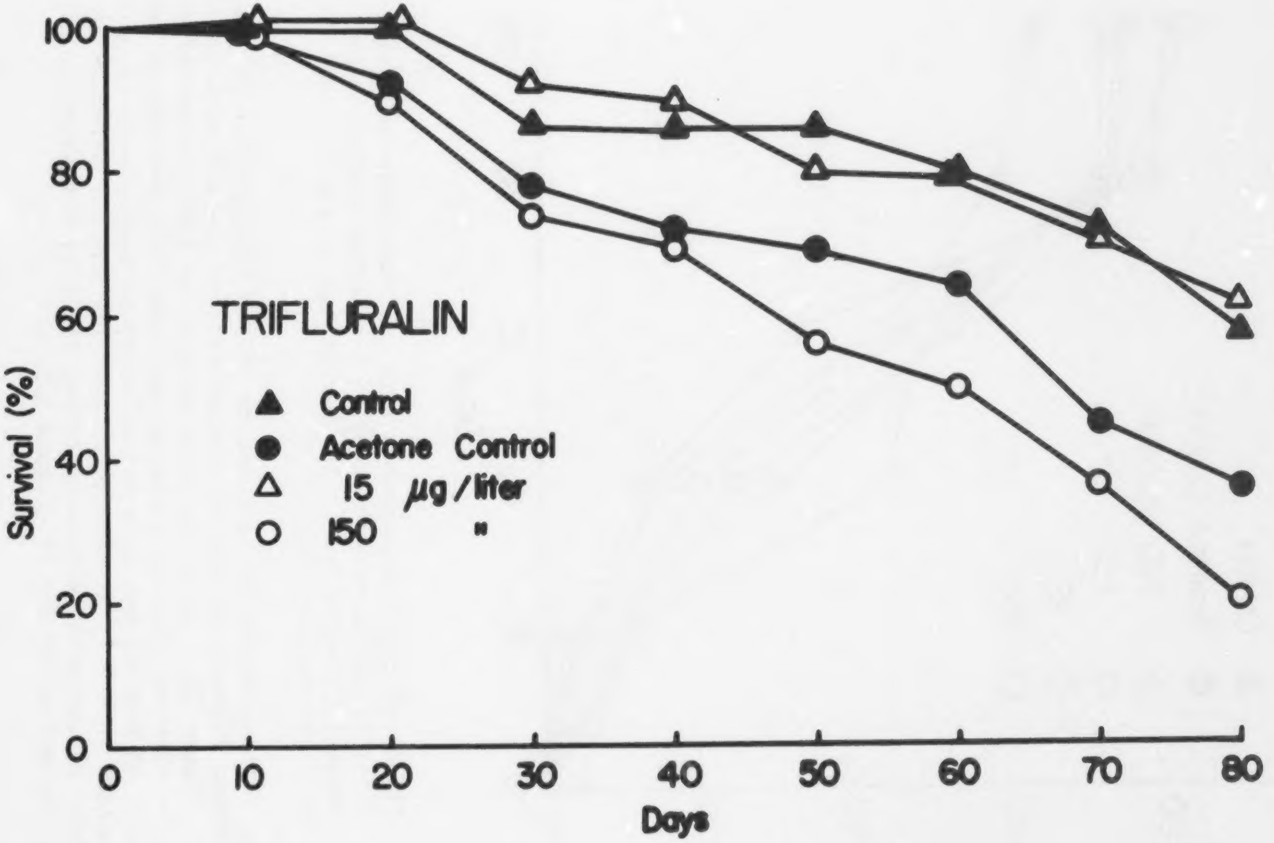


Figure 44. Effect of continuous exposure to trifluralin on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs.

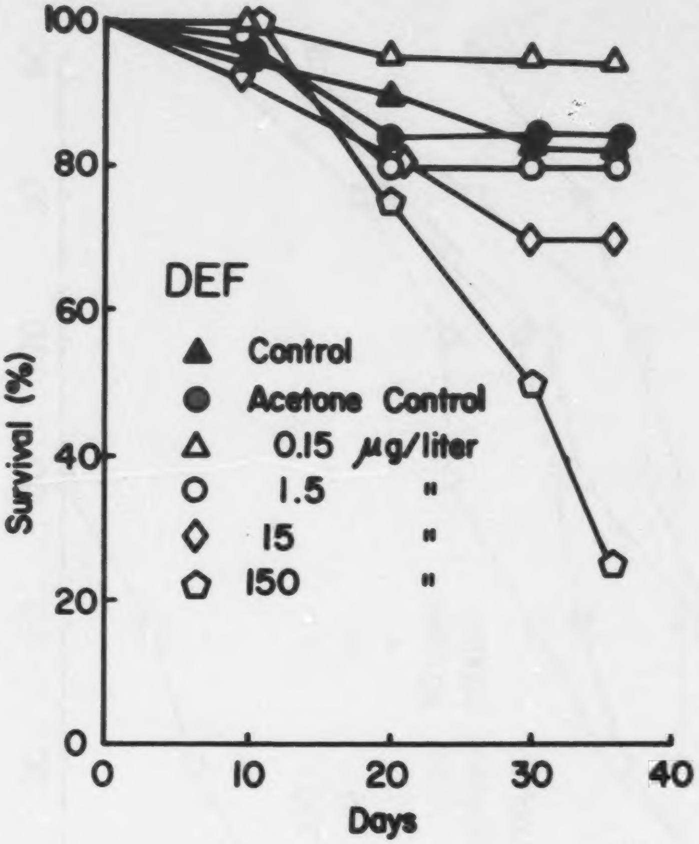


Figure 45. Effect of continuous exposure to DEF on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs.

In the second experiment, crabs exposed to propanil at 80 µg/liter survived as well as controls, at least until day 60, but the mortality rate after this time increased substantially. Propanil did not delay the time to the first molt in the first juvenile chronic experiment (Figure 50). Juvenile crabs exposed to 10,000 µg/liter 2,4-D in the first experiment exhibited poorer survival after day 10 than controls and crabs exposed to low 2,4-D concentrations (Figure 51), but molting exhibited a tendency to be advanced rather than delayed in this group (Figure 52). In the second juvenile chronic experiment 2,4-D had no effect on survival of crabs at either 1,000 or 10,000 µg/liter (Figure 53).

In long-term experiments with adult crabs, neither trifluralin nor 2,4-D acid at concentrations as high as 100 µ/liter and 10,000 µg/liter, respectively, affected survival (Figures 54 and 55). Adult crabs exposed

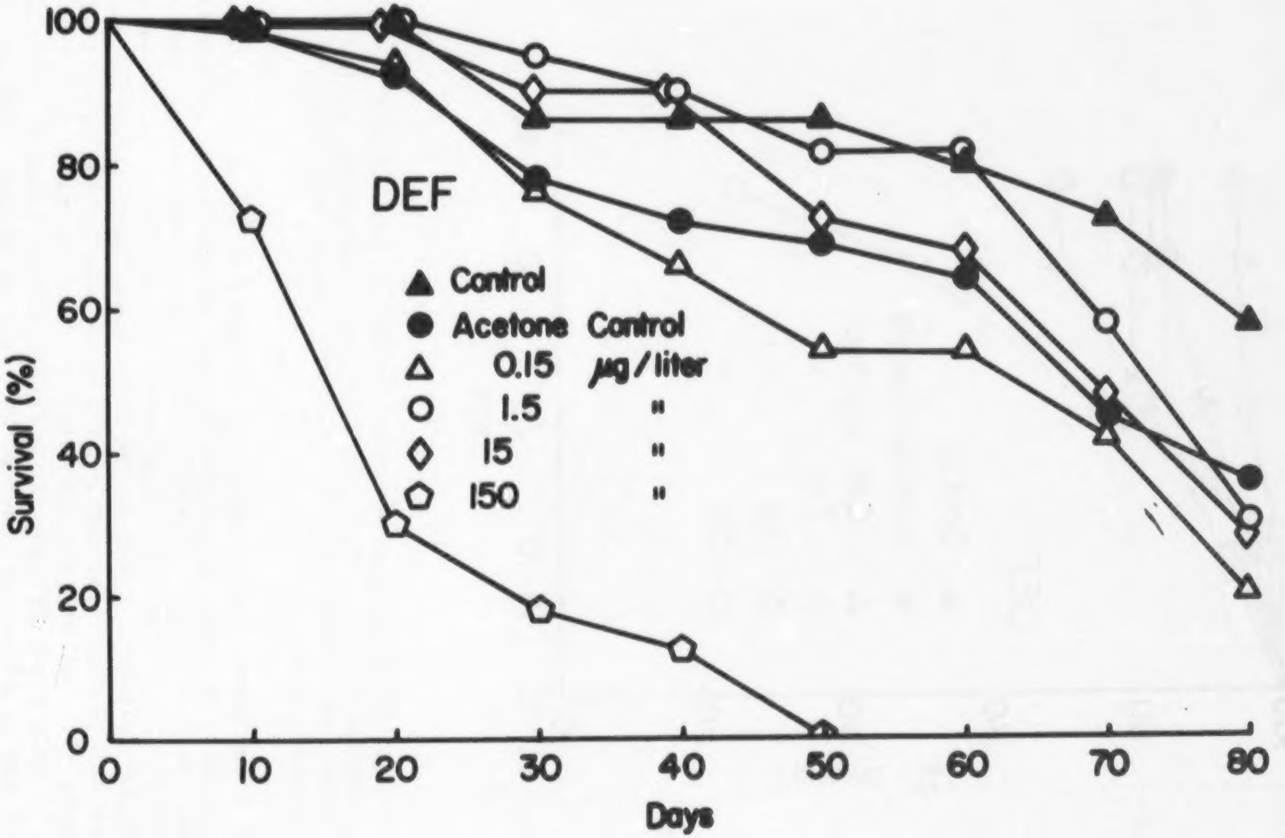


Figure 46. Effect of continuous exposure to DEF on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs.

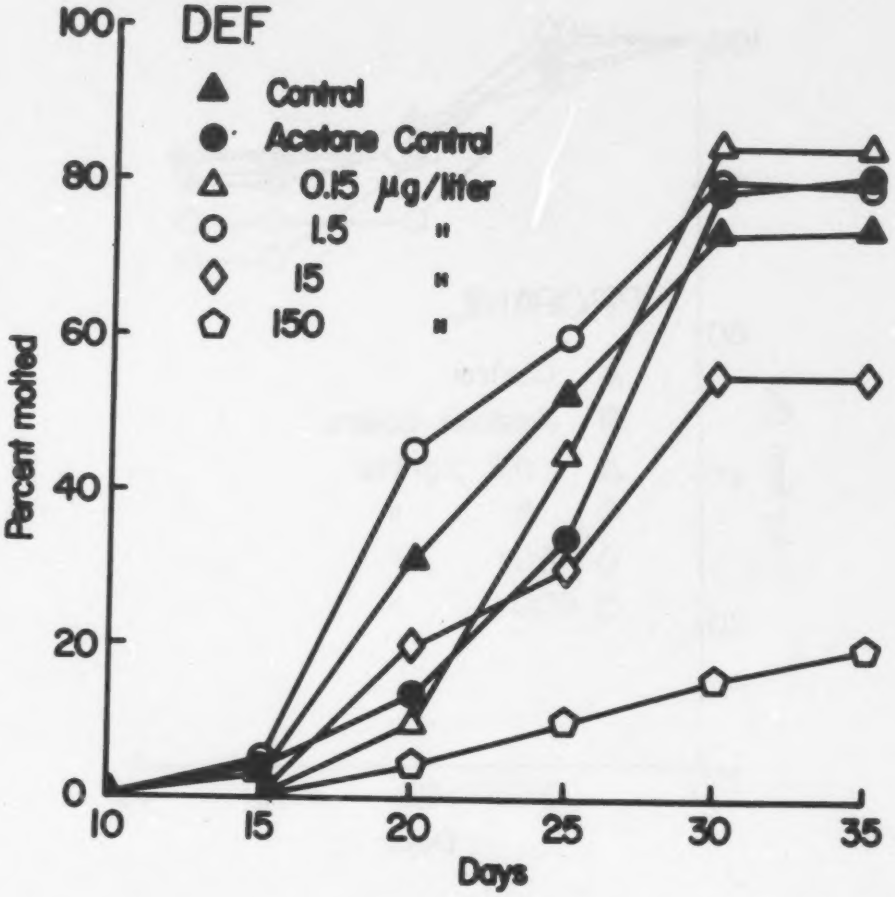


Figure 47. Effect of continuous exposure to DEF on molting of first instar Dungeness crabs to the second instar. The molt success is expressed as the percentage of the original number of first instar crabs.

chronically to DEF showed reduced survival both at 200 and 2,000 µg/liter when compared with controls (Figure 56). The median survival time at 2,000 µg/liter was about 32 days; at 200 µg/liter, about 50 days. Propanil exposure of 4,000 µg/liter reduced survival of adult crabs compared with controls, but concentrations of 400 µg/liter and lower had no effect (Figure 57). Even at 4,000 µg/liter, half of the animals survived until the end of the test on day 85.

Relatively little data are available on the toxicity of DEF to aquatic organisms. The 96-hr LC50 for the freshwater crustacean, *Gammarus lacustris*, was found to be 100 µg/liter by Sanders (1969) and the 48-hr EC50 for mortality or paralysis of the marine shrimp *Penaeus aztecus* was 28 µg/liter (Butler, 1965). In 96-hr tests the concentration of DEF causing a 50%

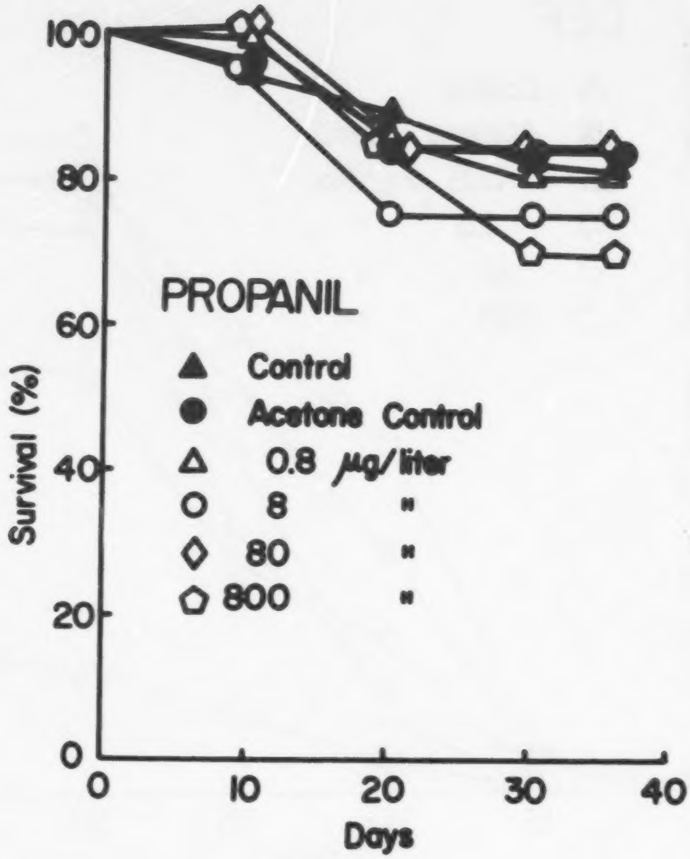


Figure 48. Effect of continuous exposure to propanil on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs.

reduction in shell growth of the oyster Crassostrea virginica was 100 µg/liter; for Leiostomus xanthurus, a marine fish, the 48-hr LC50 was 240 µg/liter (Butler, 1965). We have found that DEF has a comparable toxicity to first stage juvenile crabs; the 96-hr LC50 for this stage is 190 µg/liter (Table 19). In long-term tests survival was affected upon exposure to 150 µg/liter, but not by exposure to 15 µg/liter (Figures 45 and 46). Adult Dungeness crabs were substantially more resistant to this herbicide. We were not able to demonstrate a 50% mortality of this stage during 96 hours of exposure to 1,000 µg/liter, but survival of adult crabs was reduced during chronic exposures to 200 µg/liter (Figure 56). As has been found with other pesticides, the first stage zoeae were the most sensitive to DEF of the stages tested in our experiments. The 50% toxic concentration for zoeal swimming in 96-hr was found to be only 1 µg/liter and the 96-hr LC50 was

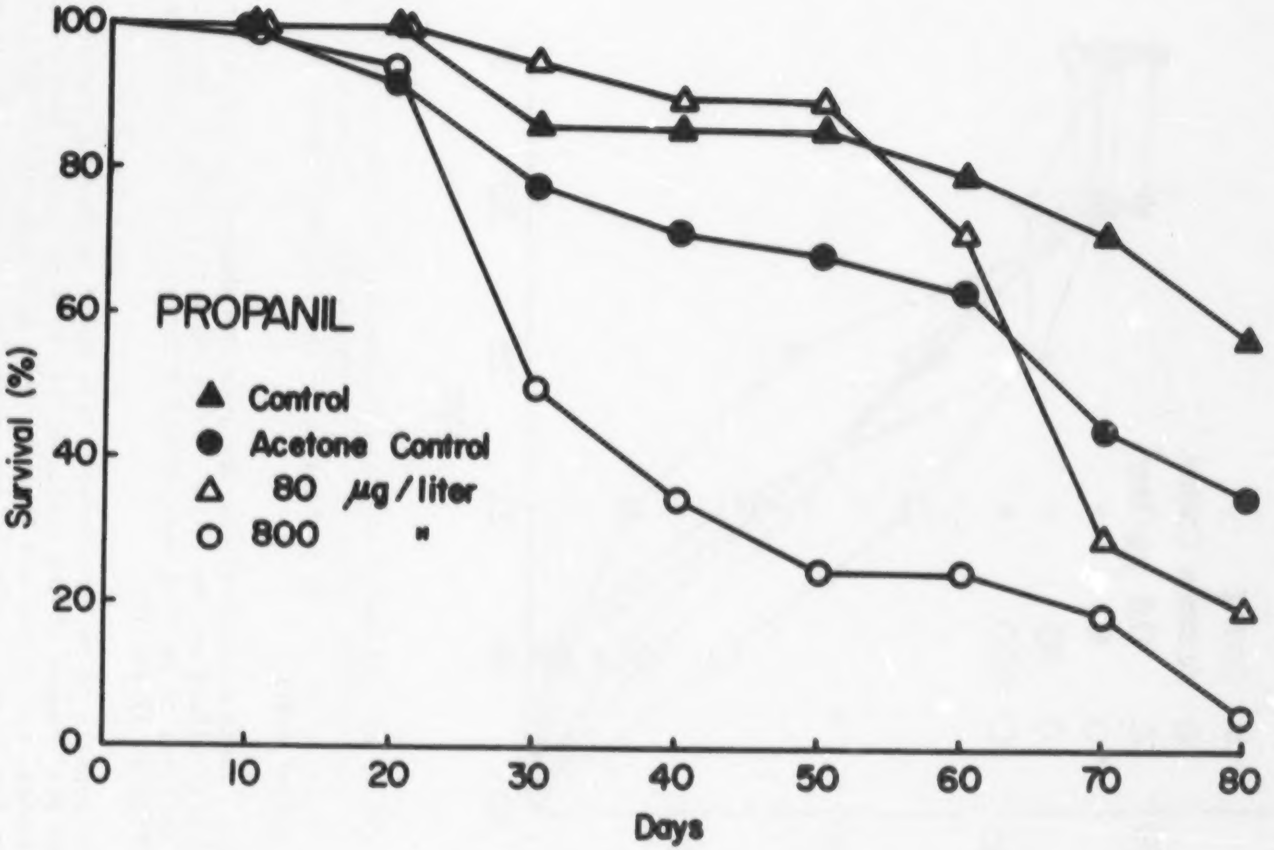


Figure 49. Effect of continuous exposure to propanil on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs.

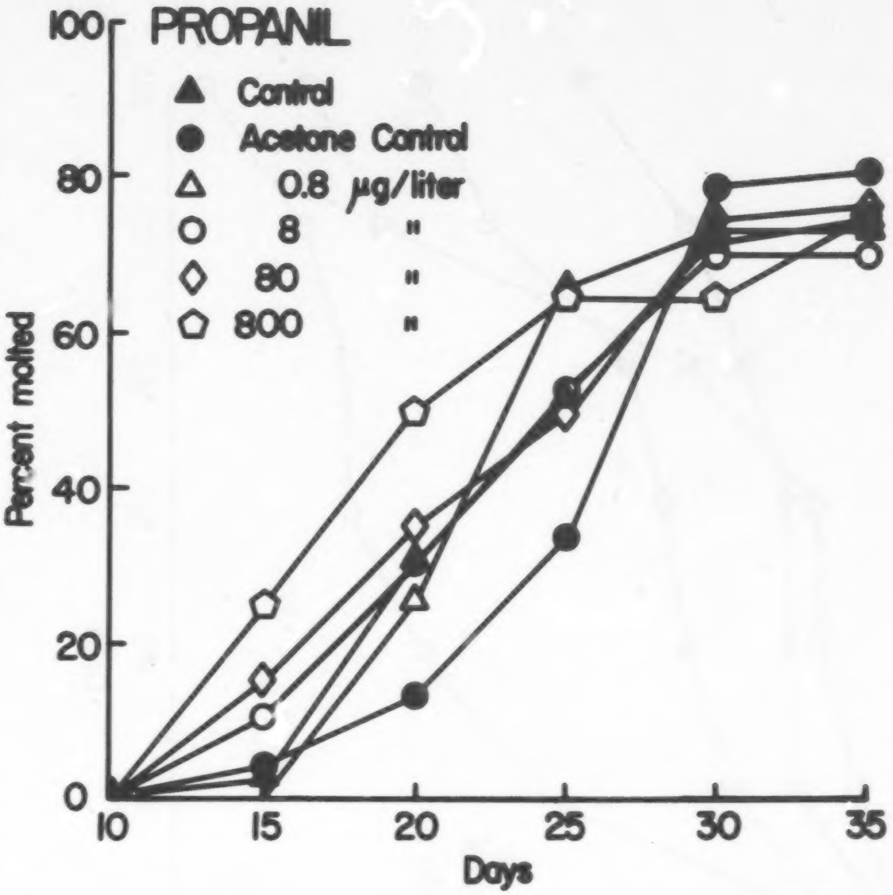


Figure 50. Effect of continuous exposure to propanil on molting of first instar Dungeness crabs to the second instar. The molt success is expressed as the percentage of the original number of first instar crabs.

6.6 µg/liter. These concentrations represent the lowest concentrations having an adverse effect on the larvae since we were unable to demonstrate that either survival or molting were affected during long-term exposure to 4 µg/liter (Figures 37 and 39).

Propanil appears to be considerably less toxic to aquatic organisms than DEF. We are unaware of any toxicological data on marine species or freshwater fishes, but Crosby and Tucker (1966) have reported a 50% effective concentration for immobilization of the freshwater crustacean Daphnia magna of 4,800 µg/liter. The 96-hr LC50 for Gammarus fasciatus is 16,000 µg/liter (Sanders, 1970). These observations are in accord with our finding on the acute toxicity of propanil to Dungeness crabs. The 96-hr LC50 for technical propanil to first instar zoeae is 7,300 µg/liter and to adult crabs exceeds

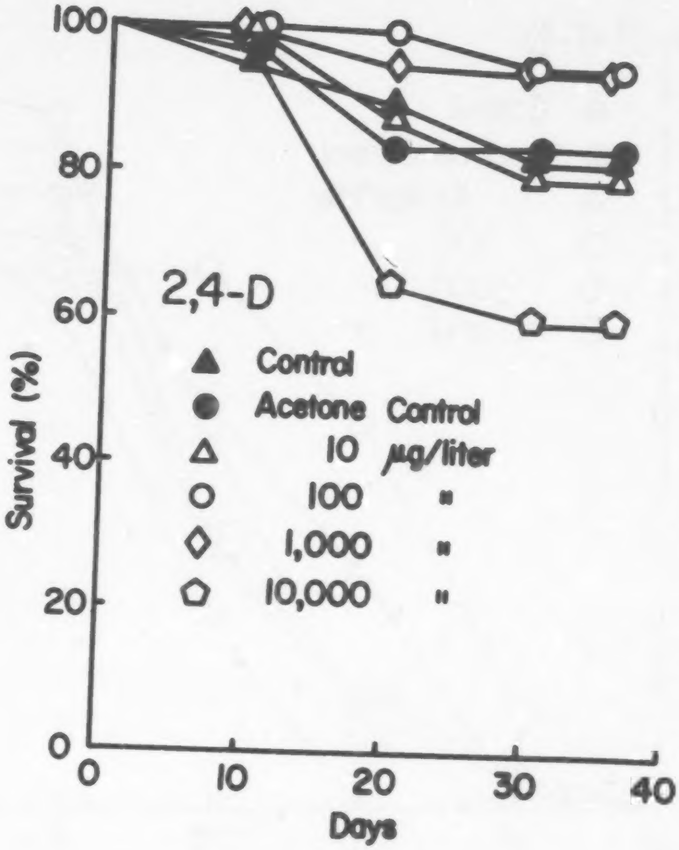


Figure 51. Effect of continuous exposure to 2,4-D on survival of juvenile Dungeness crabs. Data are from the first juvenile chronic experiment which was initiated with newly metamorphosed first instar crabs.

26,000 µg/liter (Table 19). As evidenced by reduced survival, toxic concentrations of propanil for zoeae, juveniles and adults in chronic exposure experiments were 800, 800 and 4,000 µg/liter, respectively (Figures 35, 49 and 57). For the same stages, no toxic manifestations of any kind were observed at concentrations of 80, 80 and 400 µg/liter.

The effect of trifluralin on aquatic organisms has now been rather extensively studied (Cope, 1965; Liu and Lee, 1975; Macek et al., 1969; Parka and Worth, 1965; Sanders, 1969 and 1970; Sanders and Cope, 1966, and 1968; and Walsh, 1972). The reported toxicities range from 14 to 50,000 µg/liter in acute toxicity tests of the dissolved herbicide. Teleost fish rank among the most sensitive species studied to date. The 96-hr TL50s for Salmo gairdneri and Lepomis machrochirus, each tested over a range of three temperatures, range from 42 to 210 µg/liter and 47 to 190 µg/liter, respectively, the toxicity being greatest at the higher temperatures (Macek

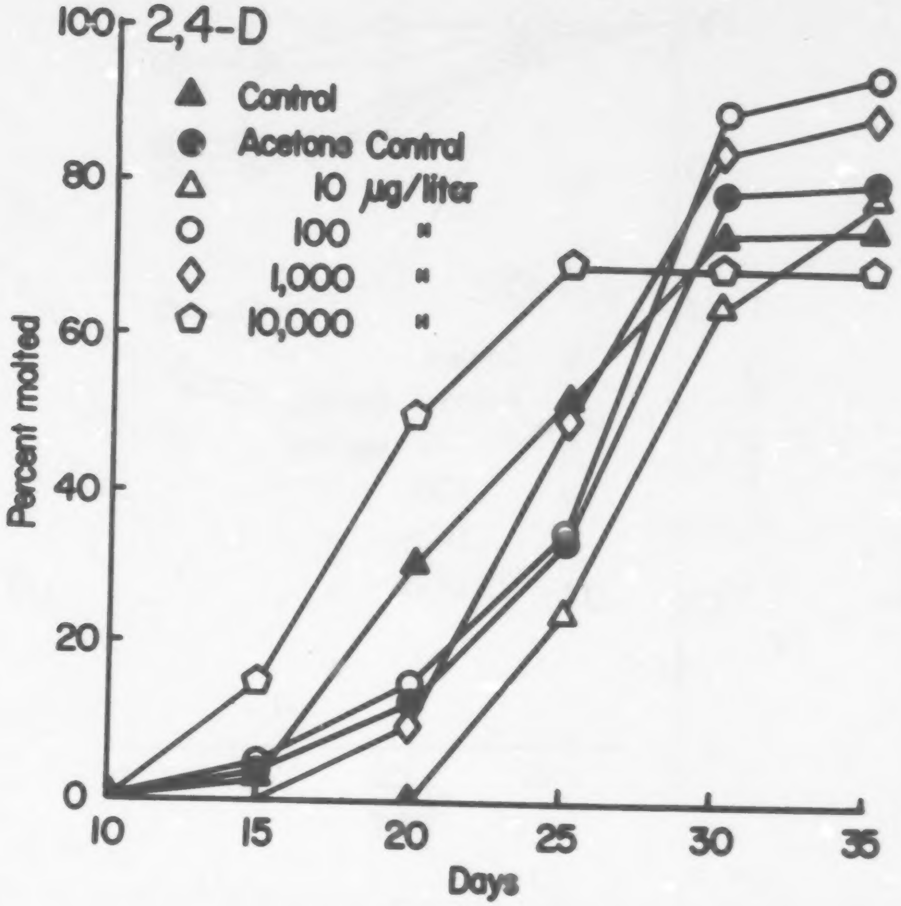


Figure 52. Effect of continuous exposure to 2,4-D on molting of first instar Dungeness crabs to the second instar. The molt success is expressed as the percentage of the original number of first instar crabs.

et al., 1969). For fingerling rainbow trout in 24-hr exposures the toxicity ranged from 14 to 210 µg/liter, and for small bluegills, from 23 to 120 µg/liter (Cope, 1965). Similar values were reported for bluegills and fathead minnows, but the 96-hr LC50 for goldfish, Carassius auratus, was 585 µg/liter (Parka and Worth, 1965). Freshwater crustaceans and insects were less sensitive to the herbicide; 48- and 96-hr LC50s ranging from 240 µg/liter for Daphnia pulex to 50,000 µg/liter for Orconectes nais (Sanders, 1969 and 1970; Sanders and Cope, 1966 and 1968). Walsh (1972) reported that the concentration of trifluralin resulting in a 50% decrease in growth of four species of marine phytoplankton was 2,500 µg/liter. The only marine animal for which toxicity data is available is the marine mussel, Mytilus edulis, (Liu and Lee, 1975). For this species 96 µg/liter reduced larval

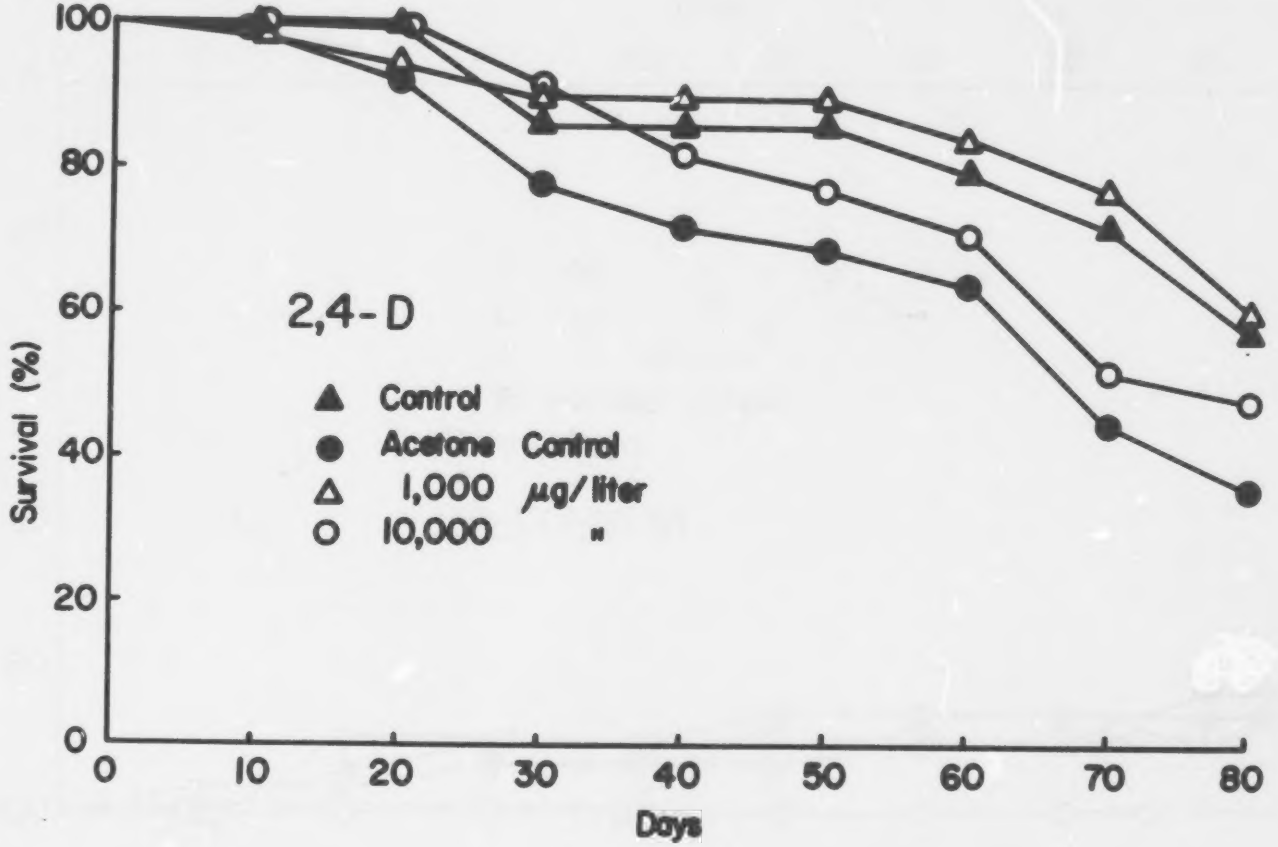


Figure 53. Effect of continuous exposure to 2,4-D on survival of juvenile Dungeness crabs. Data are from the second juvenile chronic experiment which was initiated with second and third instar crabs.

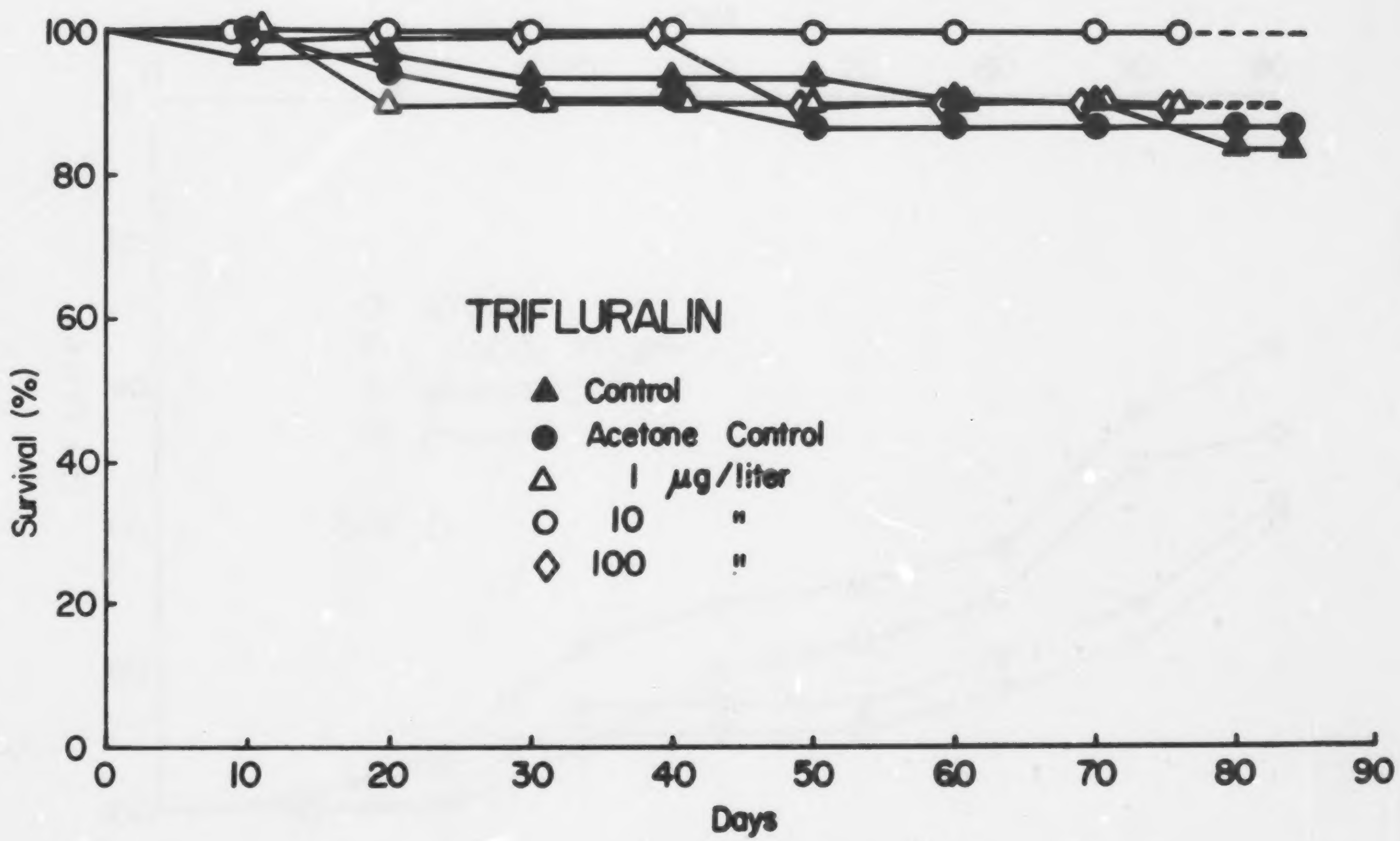


Figure 54. Effect of continuous exposure to trifluralin on survival of adult Dungeness crabs.

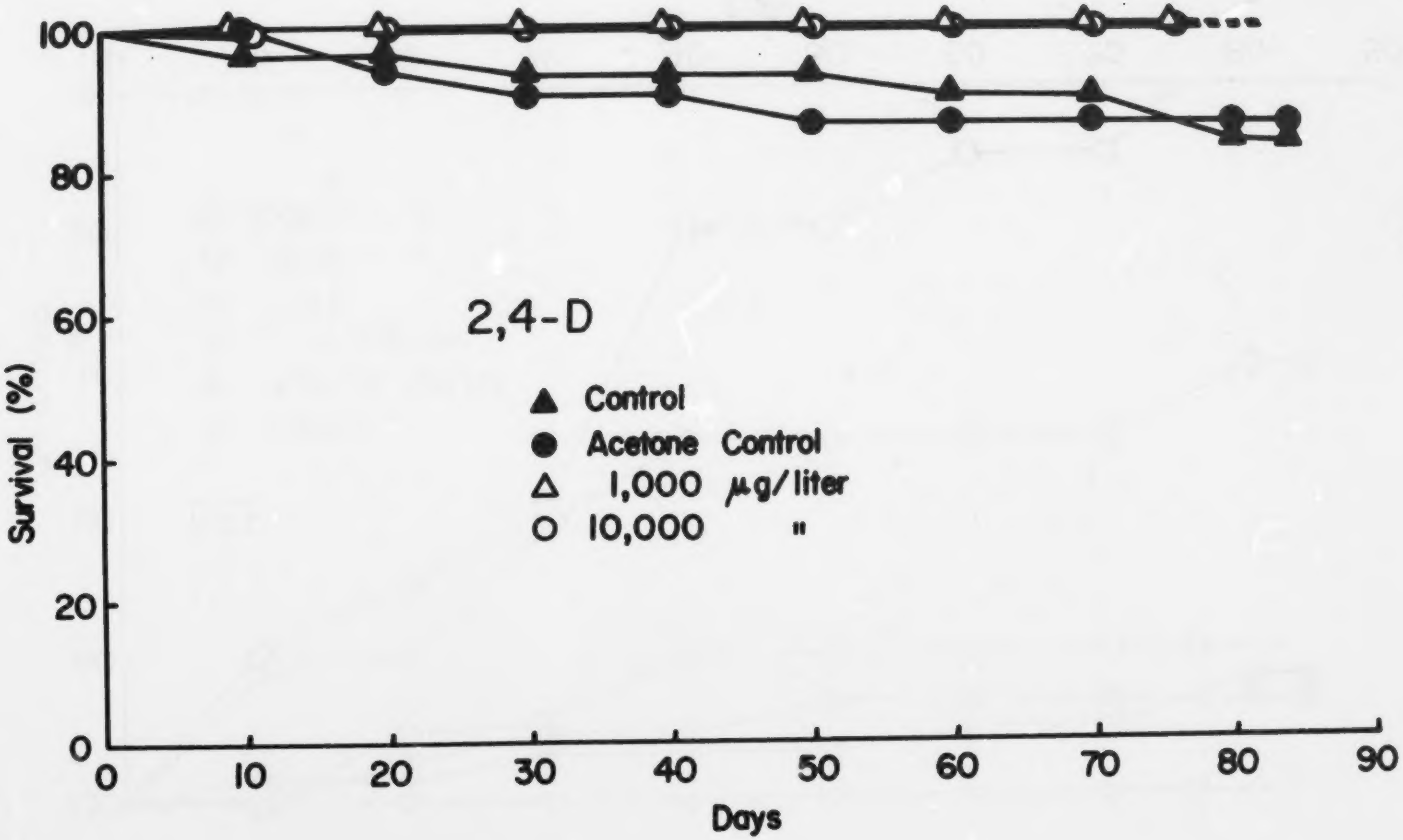


Figure 55. Effect of continuous exposure to 2,4-D on survival of adult Dungeness crabs.

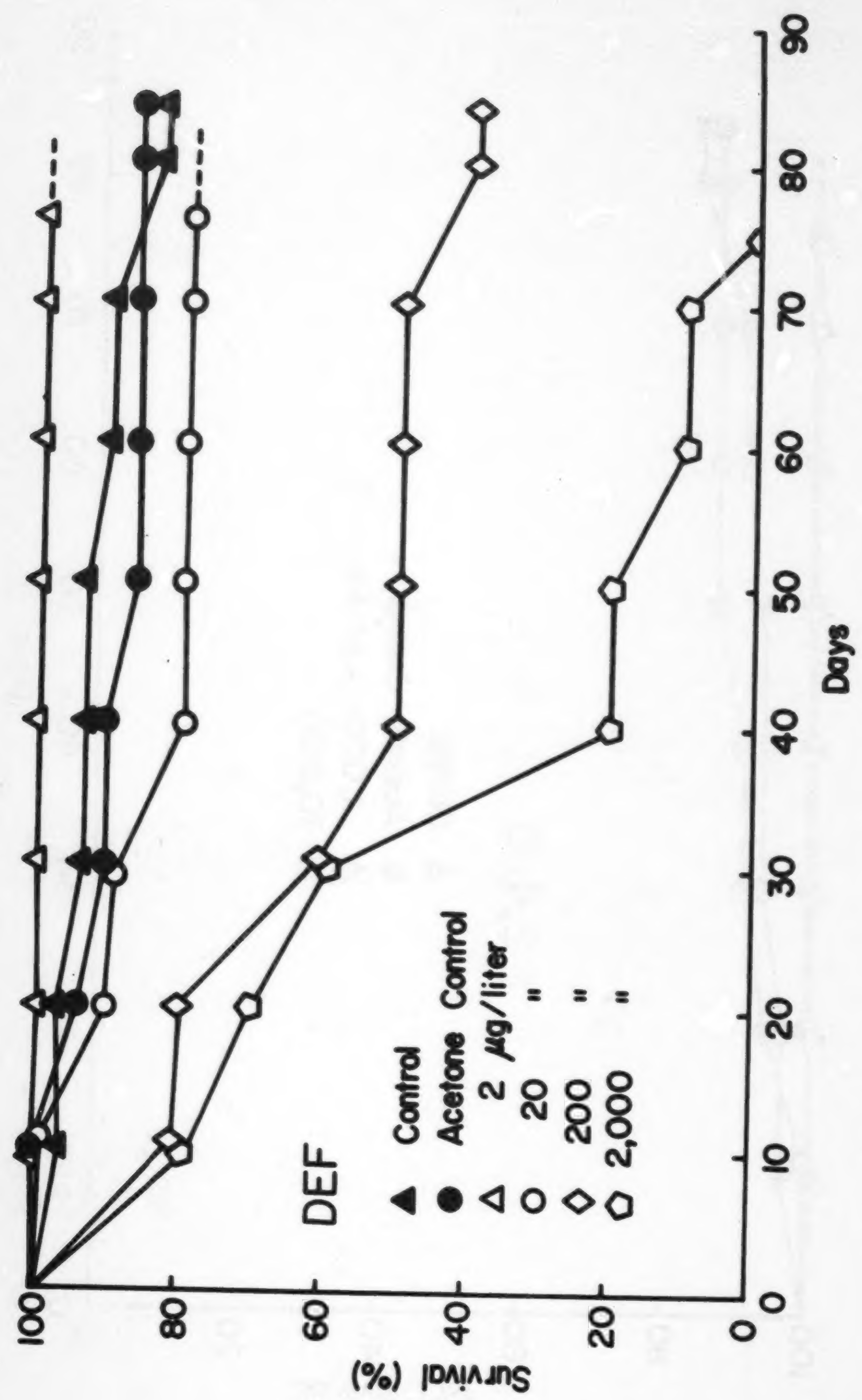


Figure 56. Effect of continuous exposure to DEF on survival of adult Dungeness crabs.

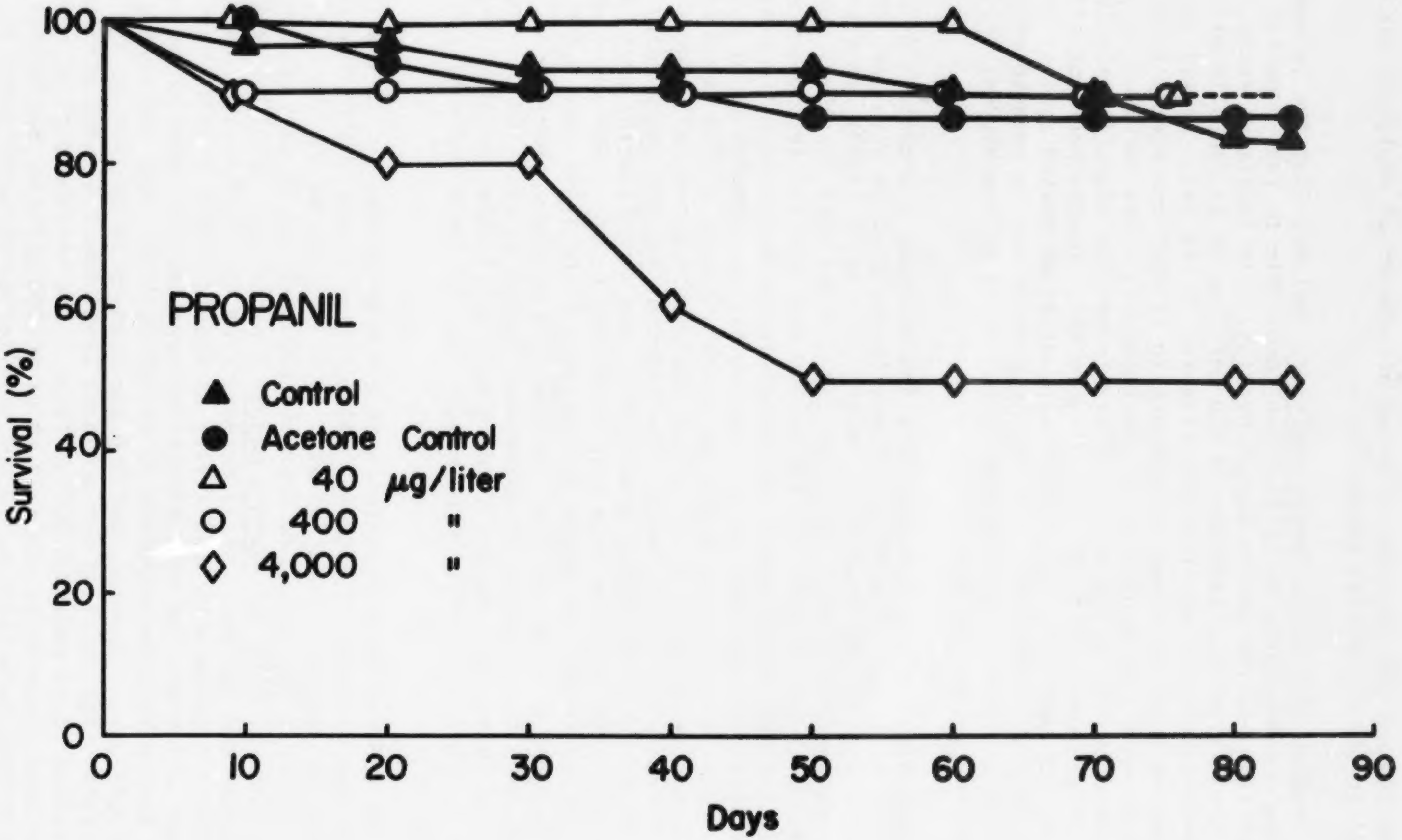


Figure 57. Effect of continuous exposure to propanil on survival of adult Dungeness crabs.

growth and 100 and 240 µg/liter affected attachment of adults to glass and was lethal to 50% of adults, respectively.

Our studies with crabs, Cancer magister, indicate that the larvae of this species has a sensitivity to trifluralin comparable to that reported for freshwater fish and the marine mussel Mytilus. In long-term tests, larvae were rapidly killed upon exposure to 150 µg/liter of trifluralin with 50% of the deaths occurring in about 5 days (Figures 30 and 34). Larval survival and molting were not affected by exposure to 15 µg/liter for up to 69 days. Older stages were less sensitive to the herbicide. The survival of juvenile crabs exposed to 150 µg/liter of trifluralin was not significantly less than controls in exposures up to 80 days (Figure 44). Adults were not affected by exposure to 100 µg/liter, the highest concentration tested against this stage, in 85 days (Figure 54). In addition there was no adverse effect on egg hatching or prezoeal development in our tests at the highest trifluralin concentration tested, 330 µg/liter (Figure 26).

An assessment of the toxicity of 2,4-D to aquatic organisms is complicated by the fact that this herbicide may be employed as a variety of esters and salts as well as the free acid. Hughes and Davis (1963), in studies with the bluegill sunfish (Lepomis machrochirus), found that the 48-hr toxicity of various formulations ranged from 800 to 840,000 µg/liter (expressed as acid equivalents). The alkanolamine and dimethylamine salts were the least toxic, and the esters were the most toxic. An emulsified formulation of 2,4-D acid was also relatively toxic at 8,000 µg/liter. Sanders (1970) found that the relative toxicity of 2,4-D formulations could vary appreciably for different species of freshwater crustaceans. For example, Daphnia magna 48-hr TL50s for the propylene glycol butyl ether ester, the dimethylamine salt, the butoxyethanol ester and the free acid were 100, 4,000, 5,600 and >100,000 µg/liter, respectively, but Gammarus fasciatus 48-hr TL50s were 2,600, 100,000, 5,900 and 3,200 µg/liter for the same series. Species differences were also noted for a series of salmonids (Meehan et al., 1974). The latter authors also found that the propylene glycol butyl ether ester was more toxic than the isooctyl ester, and the pure acid was the least toxic of the formulations tested.

Values for the aquatic toxicity of 2,4-D acid reported in the literature range from approximately 1,000 to 3,000 µg/liter (Butler, 1965 for the marine shrimp Penaeus aztecus; Meehan et al., 1974 for pink salmon fry; Sanders, 1970 for Gammarus fasciatus) to over 100,000 µg/liter (Crosby and Tucker, 1966 and Sanders, 1970 for Daphnia magna; Liu and Lee, 1975 for Mytilus edulis larvae and adults). It is evident from our studies that the Dungeness crab exhibits a comparable sensitivity to the acid form of this herbicide. The larvae, which was the most sensitive stage tested, experienced reduced survival during extended exposure to 10,000 µg/liter, but not during exposure to 1,000 µg/liter. However, molting appeared to be delayed at the latter concentration. Egg hatching and prezoeal development was not affected at concentrations up to about 33,000 µg/liter. The threshold of effects on juvenile crabs appeared to occur at about 10,000 µg/liter, and adults were not affected in long-term tests at 10,000 µg/liter or in acute tests at 100,000 µg/liter.

THE INSECTICIDES CARBOFURAN, CHLORDANE, AND MALATHION

Unlike the results previously observed with captan, methoxychlor, and the four herbicides 2,4-D, DEF, propanil and trifluralin, exposure of eggs to the insecticide carbofuran did not result in an acceleration of egg hatching (Figure 58). At insecticide concentrations ranging from 1 to 330 µg/liter, hatching success during the 24-hr test period ranged from 24 to 50% averaging 38 ± 9%. Furthermore, no trend in hatching success was noted with increasing carbofuran concentrations. The effects of carbofuran on zoeal development and on motility of developed zoeae followed the usual pattern, however motility being affected at lower concentrations of the insecticide than those that affected the development from prezoeae to zoeae (Figure 58). The 50%

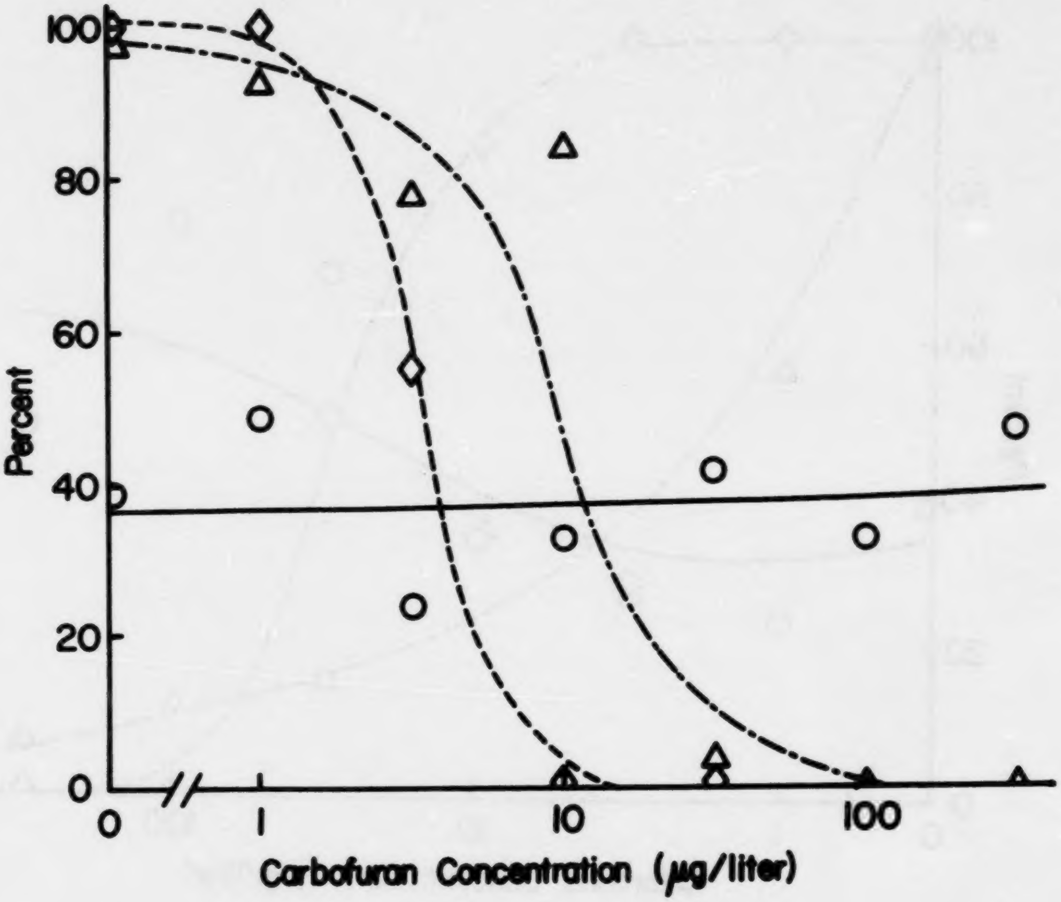


Figure 58. Percent hatch of Dungeness crab eggs (○——○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—·—△), and percent of developed first stage zoeae which are motile (◇----◇) as a function of carbofuran concentration during a 24-hr exposure period.

effective concentration for zoeal motility was approximately 3.3 µg/liter, while that for zoeal development was approximately 10 µg/liter.

The results of chlordane treatment on egg hatching, zoeal development, and zoeal motility were the most unusual. Egg hatching was unaffected or even slightly depressed at the lower test concentrations, 1 to 10 µg/liter, but appeared to be accelerated at higher insecticide concentrations, 33 to 330 µg/liter (Figure 59). The most unusual response was the relationship between zoeal development and zoeal motility. Unlike any of the other pesticides, development of prezoeae into the first zoeal stage was inhibited at a lower concentration, approximately 2 to 3 µg/liter, than the motility of those zoeae that developed, approximately 33 µg/liter (Figure 59).

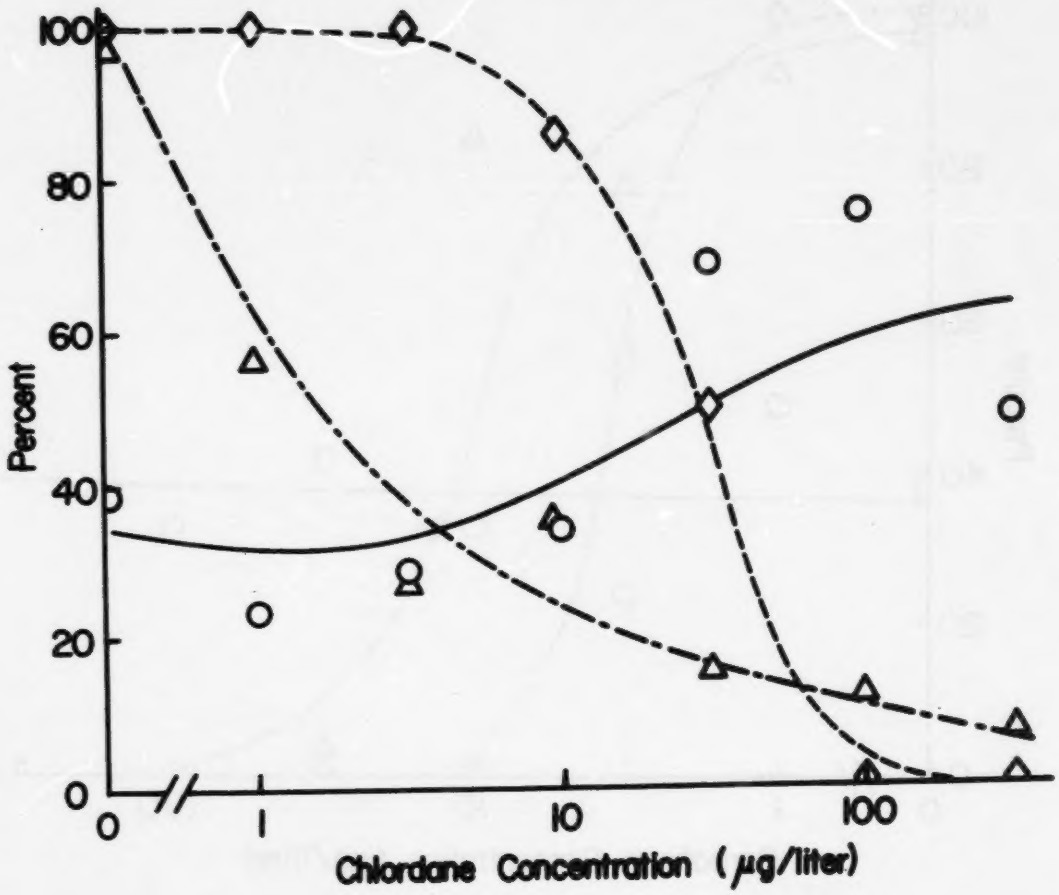


Figure 59. Percent hatch of Dungeness crab eggs (○———○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—·—△), and percent of developed first stage zoeae which are motile (◇----◇) as a function of chlordane concentration during a 24 hr-exposure period.

The pattern of effects of malathion on these early developmental stages was like that seen with captan, methoxychlor, DEF and propanil. All concentrations of the organophosphate from 0.33 to 100 μg/liter resulted in accelerated egg hatching compared to the controls; the average hatch success of the insecticide-treated eggs being about 70% (Figure 60). The success of development of prezoeae into first stage zoeae was 90% or higher even at the highest pesticide treatment, 100 μg/liter, but zoeal motility was 50% affected at only 11 to 12 μg/liter of malathion (Figure 60).

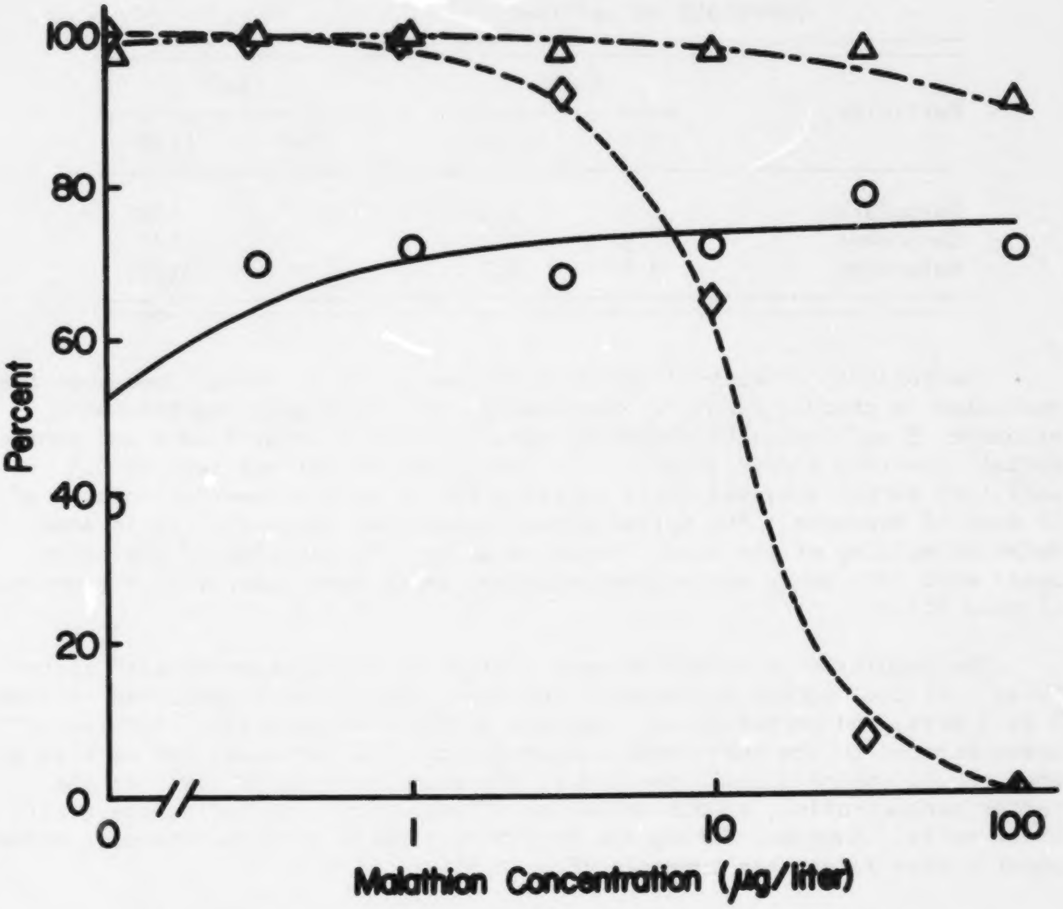


Figure 60. Percent hatch of Dungeness crab eggs (○——○), percent development of hatched crab larvae through the prezoeal to the first zoeal stage (△—-—△), and percent of developed first stage zoeae which are motile (◇----◇) as a function of malathion concentration during a 24-hr exposure period.

The acute toxicity of all three insecticides for zoeae was similar (Table 20). The 96-hr LC50 for carbofuran was 2.5 μg/liter. Based on the sublethal criterion, inhibition of swimming, the 50% effective concentration was 1.5 μg/liter of the same insecticide. The LC50 for chlordane exceeded the highest concentration tested, 10 μg/liter, but the EC50 was 1.3 μg/liter, a value similar to that for carbofuran. Comparable data for malathion were 1.2 and 0.4 μg/liter, respectively.

TABLE 20. ACUTE TOXICITY OF INSECTICIDES TO FIRST INSTAR ZOEAE AND ADULT CRABS IN 96-HR TESTS. DATA ARE EXPRESSED IN μg/liter

Pesticide	Zoeae		Adult	
	EC50	LC50	EC50	LC50
Carbofuran	1.5	2.5	-	190.
Chlordane	1.3	>10.	-	220.
Malathion	0.4	1.2	-	1,330.

The toxicity of each of the insecticides to first instar zoeae was also evaluated in chronic exposure experiments. At the highest carbofuran exposure, 5 μg/liter, 50% mortality occurred within about 4 days and complete mortality within 7 days (Figure 61). No lethal effect was seen at 0.5 μg/liter; larval survival still exceeded 80% at this concentration even after 50 days of exposure. The latter concentration may have resulted in some delay of molting of the zoeal stages, however. By the time of the third zoeal molt this group molted approximately three days later than the controls (Figure 62).

The results with malathion were similar to those observed with carbofuran. At the highest exposure, 2 μg/liter, 50% mortality occurred in from 4 to 5 days, and mortality was complete by day 6 (Figure 63). Survival of zoeae exposed to the next lower concentration, 0.2 μg/liter, was as high as that of the controls until the end of the experiment on day 69. At the latter concentration, malathion had no effect on molting during the first three molts. However, during the fourth molt zoeae at this treatment molted about 3 days later than controls (Figure 64).

As in the egg tests, chlordane produced unusual results in the long-term zoeal experiment. At the highest concentration, 15 μg/liter, the survivorship curve is almost identical with those of the highest concentrations of carbofuran and malathion, i.e. 50% mortality occurred on about day 4, and 100% mortality by day 6 (Figure 65). Compared with controls, however, considerable mortality occurred even at 10% and 1% of this highest chlordane concentration. At an exposure of 1.5 μg/liter, 50% mortality occurred between day 8 and day 9, and 100% mortality on day 20. At only 0.15 μg/liter chlordane, 50% mortality was noted on day 37 compared with survival of more

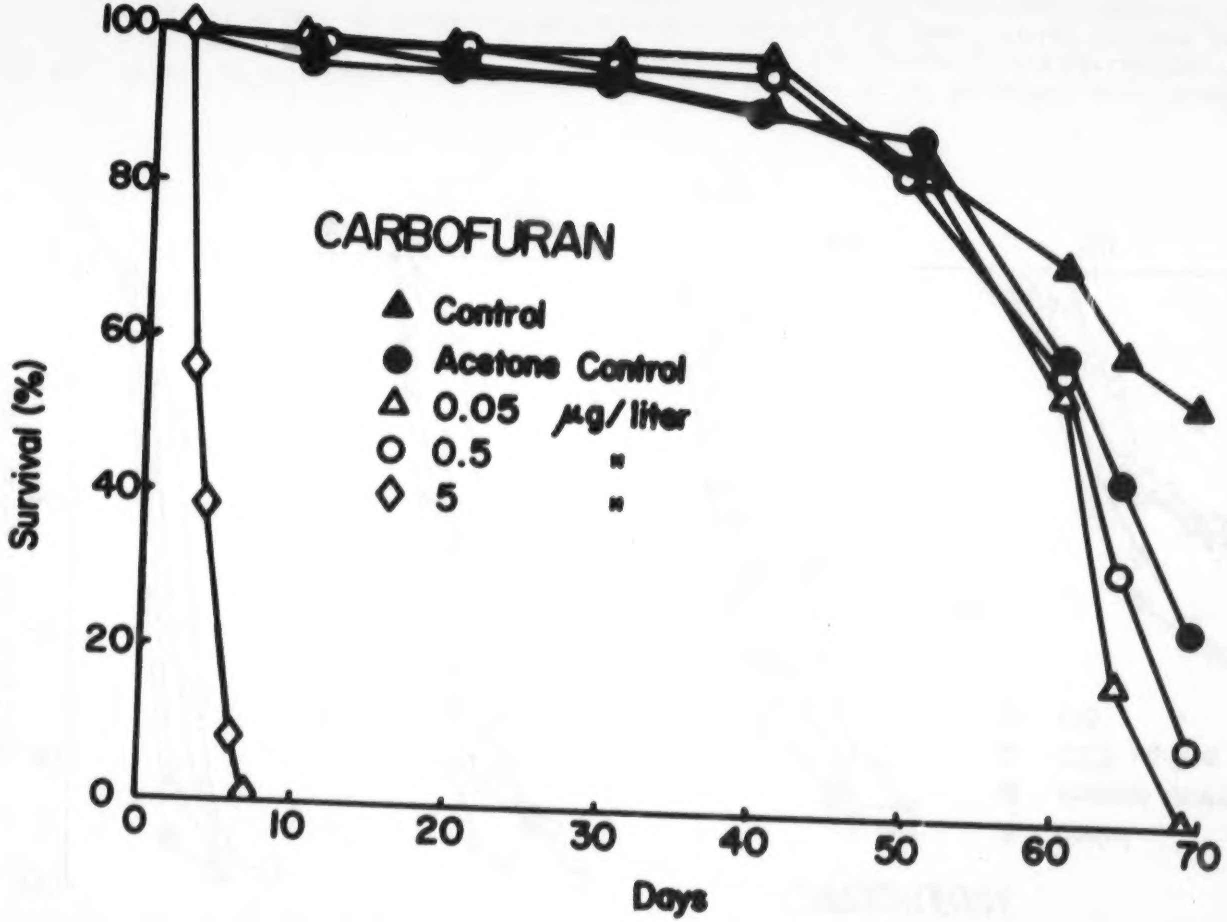


Figure 61. Effect of continuous exposure to carbofuran on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

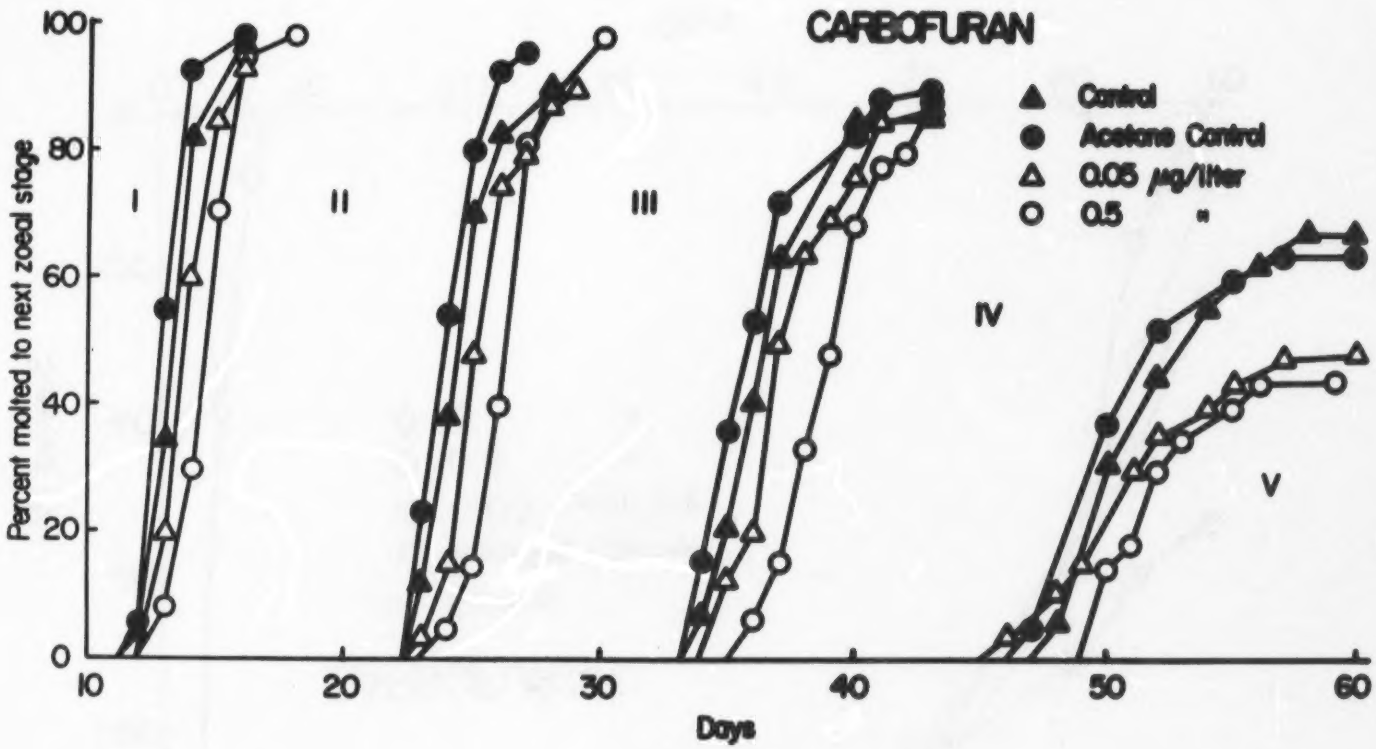


Figure 62. Effect of continuous exposure to carbofuran on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success of each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

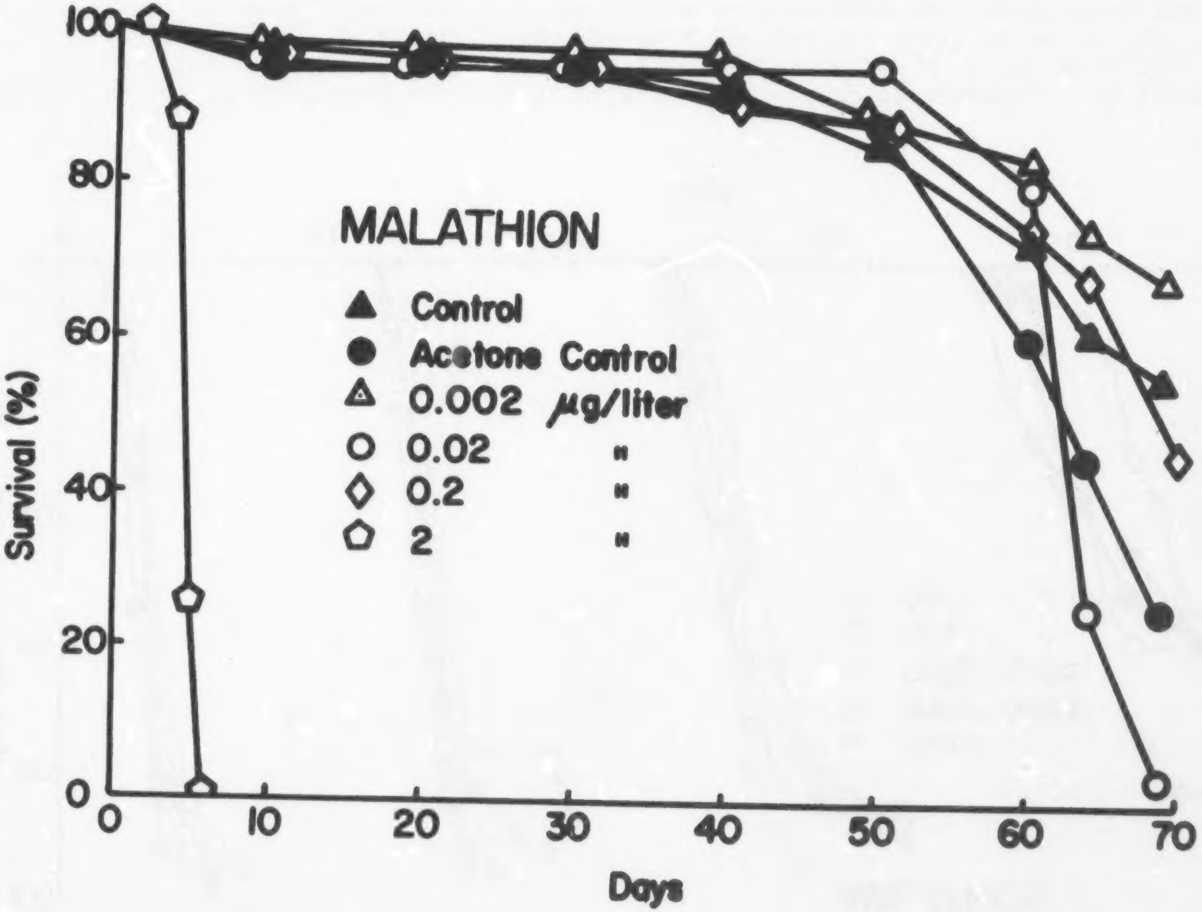


Figure 63. Effect of continuous exposure to malathion on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

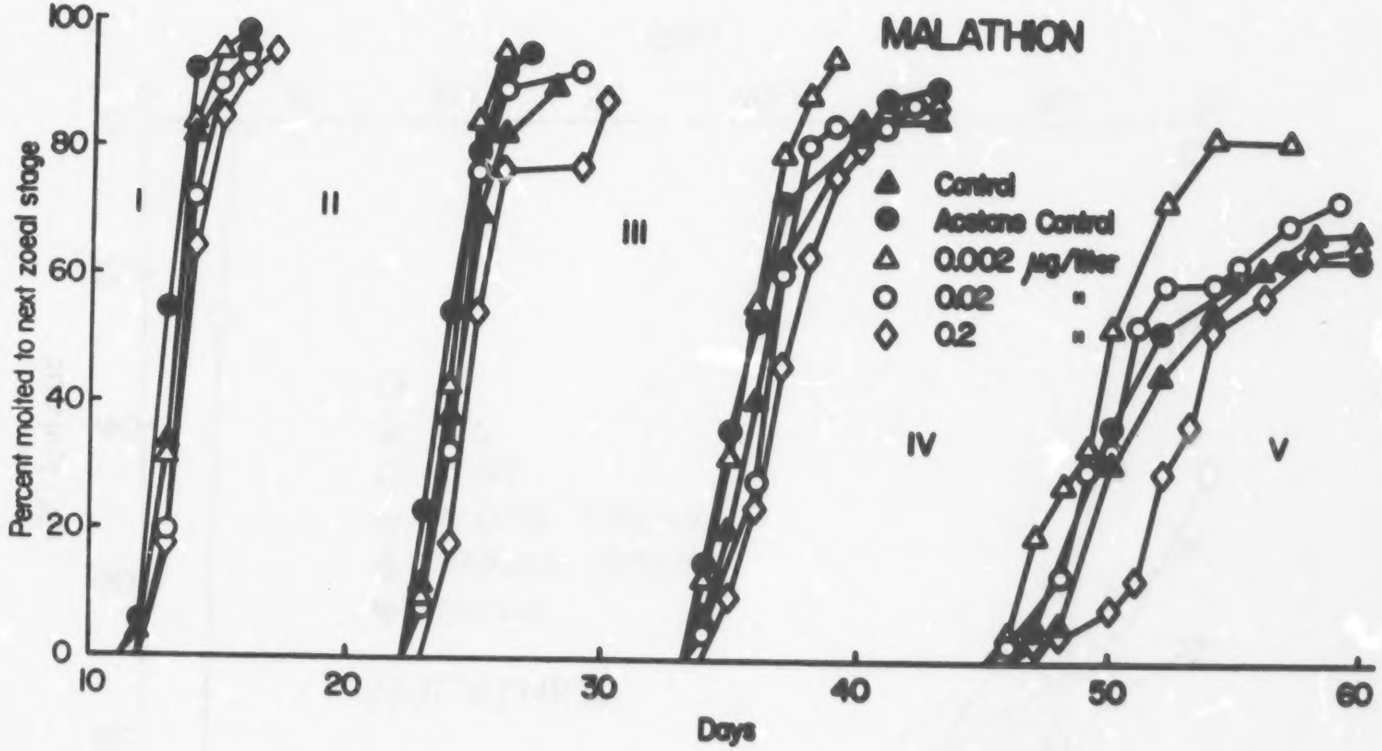


Figure 64. Effect of continuous exposure to malathion on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success of each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

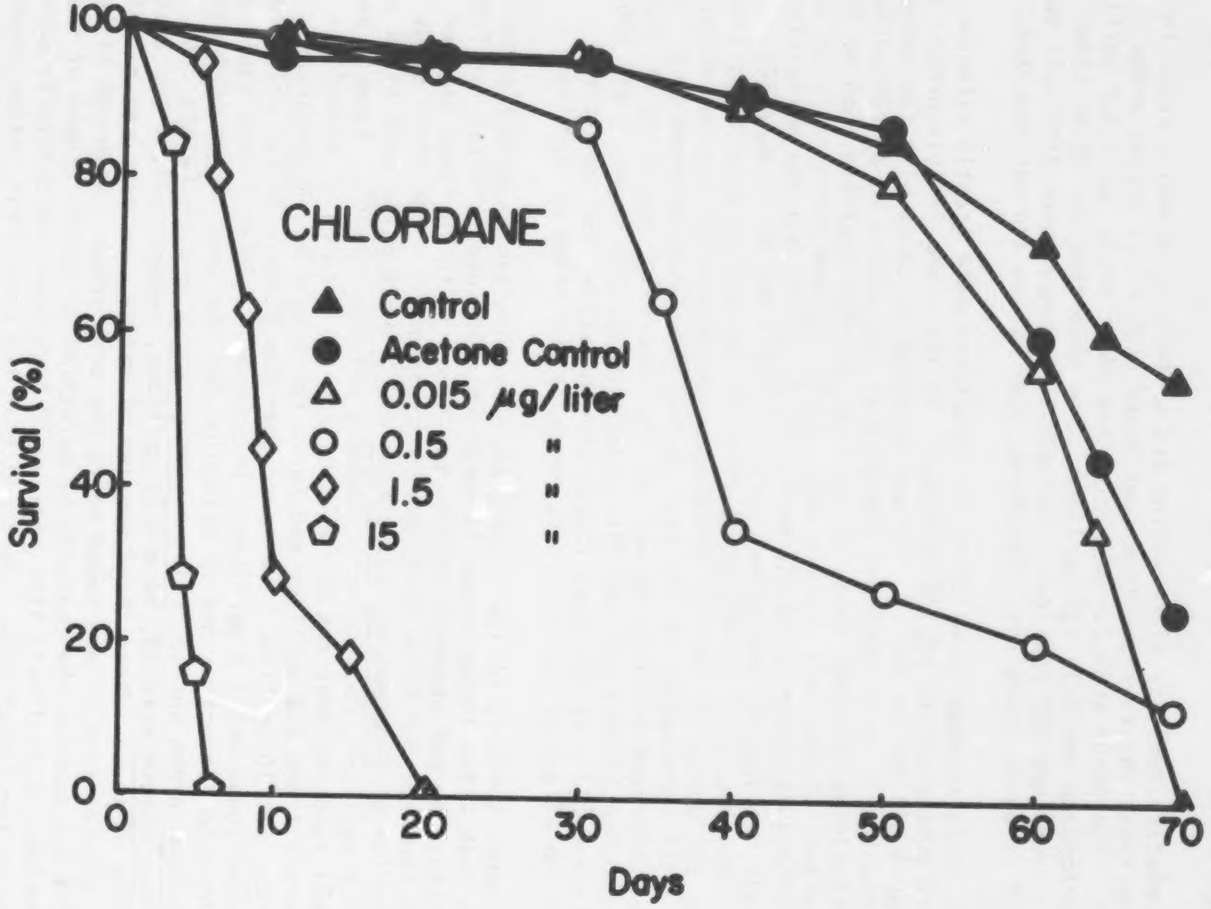


Figure 65. Effect of continuous exposure to chlordane on survival of Dungeness crab zoeae. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

than 90% of the control larvae. Larval molting was also apparently affected at this concentration (Figure 66). Survival of crabs exposed to 0.015 µg/liter was not different than that of control animals at any time during the chronic tests. Furthermore, there was no evidence that chlordane at this concentration influenced the time of molting compared with controls (Figure 66).

The relative toxicity of malathion and carbofuran to adult crabs in 96-hr acute tests was reversed from that noted with first instar zoeae (Table 20). The 96-hr LC50 for malathion and adult crabs was 1,330 µg/liter, but for carbofuran was only 190 µg/liter. For chlordane the 96-hr LC50 with adult crabs was 220 µg/liter. Sublethal responses were difficult to distinguish in adult crabs and, therefore, EC50 values are not reported.

Adult crabs exposed chronically to carbofuran were greatly affected by an exposure concentration of 250 µg/liter. Of the crabs in this group, 50% died by day 4 and 90% by day 10. As was observed in tests with the zoeae exposed chronically to carbofuran, exposure of the adults to a concentration of the insecticide 10 times less, 25 µg/liter in this instance, had no discernible effect on survival (Figure 67). Crabs exposed chronically to 100 µg/liter chlordane experienced 50% mortality by day 8, but the mortality rate subsequently declined as one animal survived until day 45 (Figure 68). High mortality was also noted in the group of crabs exposed to only 10 µg/liter chlordane; 50% mortality in this group occurred on day 23, but one animal survived until termination of the test on day 90. Crabs exposed to 0.1 and 1 µg/liter chlordane survived as well as the controls. Mortality of crabs exposed chronically to 1,500 µg/liter malathion was 50% by day 3 and 100% by day 12 (Figure 69). At the next lowest concentration, 150 µg/liter malathion, no effect of the pesticide exposure was noted on survival.

The acute toxicity of the organophosphate insecticide malathion to Dungeness crab first instar zoeae is comparable to that reported for freshwater crustaceans and stonefly naiads, but about 30 to 70 times greater than that reported for three adult marine crustaceans. The 48-hr EC50s for immobilization of Simocephalus serrulatus and Daphnia pulex were found to be 3.5 and 1.8 µg/liter, respectively (Sanders and Cope, 1966). Sanders and Cope (1968) reported that the 96-hr LC50s for two species of stonefly naiads were 1.1 and 2.8 µg/liter and for a third species, Pteronarces californica, was 10 µg/liter. We found that the 96-hr LC50 for malathion to first instar zoeae was 1.2 µg/liter. In contrast, Eisler (1969) reported that 96-hr LC50 concentrations of malathion for the sand shrimp, Crangon septemspinosa, grass shrimp, Palaemonetes vulgaris and the hermit crab, Pagurus longicarpus were 33, 82 and 83 µg/liter, respectively. The lesser sensitivity of these latter three species of marine crustacea may be related to their greater size or age compared to the crab zoeae. Although Eisler (1969) did not examine the toxicity of malathion to larval stages of these three species, it is likely that they would have been substantially more sensitive to the insecticide. We found that the crab first instar zoeae were more than 1100 times as sensitive to malathion as the adult crabs (Table 20).

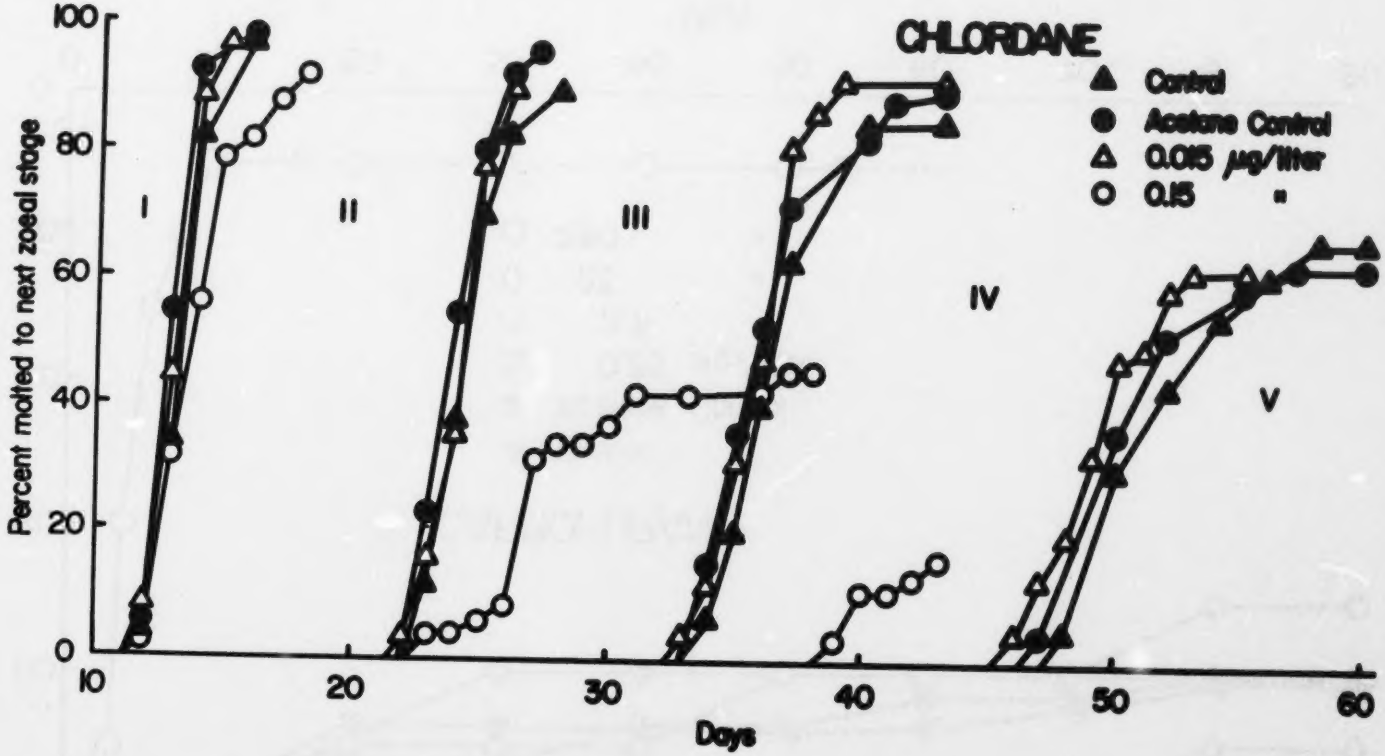


Figure 66. Effect of continuous exposure to chlordane on molting of Dungeness crab zoeae beginning with the first zoeal molt. The molt success of each stage is the percentage of the original number of zoeae. Roman numerals indicate the zoeal instar at each stage in the zoeal sequence. Data are from the second zoeal chronic experiment conducted during the spring of 1974.

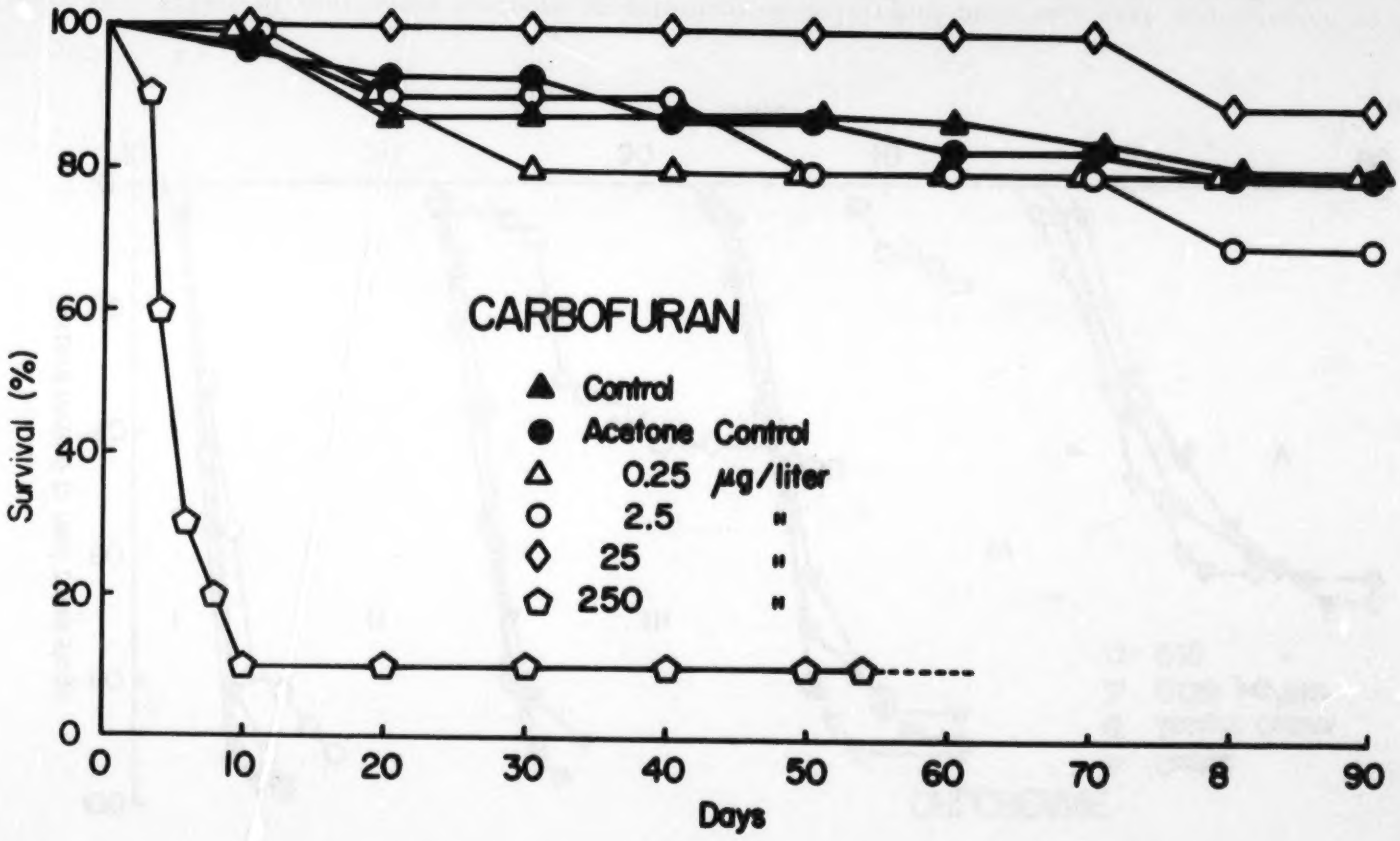


Figure 67. Effect of continuous exposure to carbofuran on survival of adult Dungeness crabs.

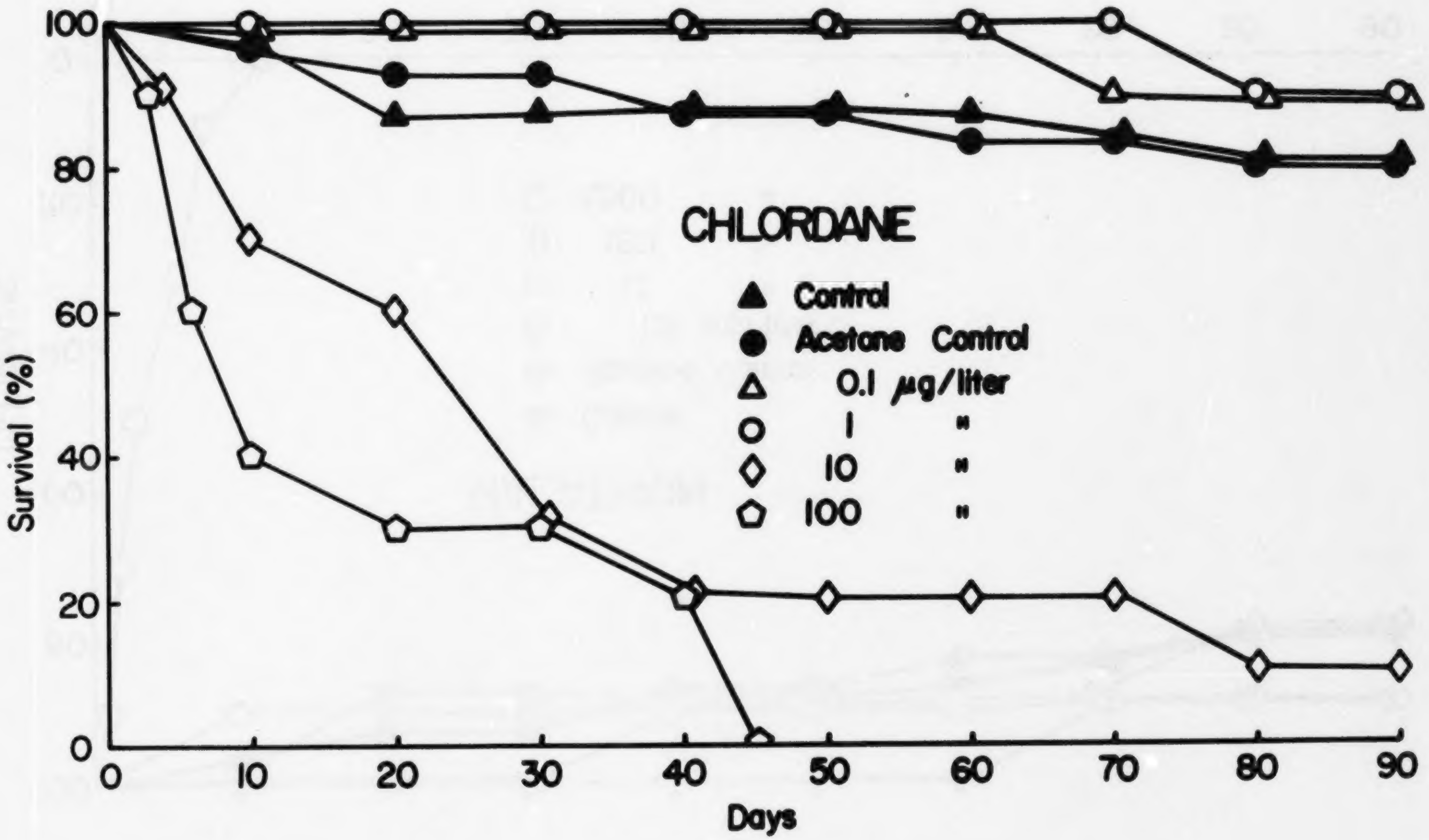


Figure 68. Effect of continuous exposure to chlordane on survival of adult Dungeness crabs.

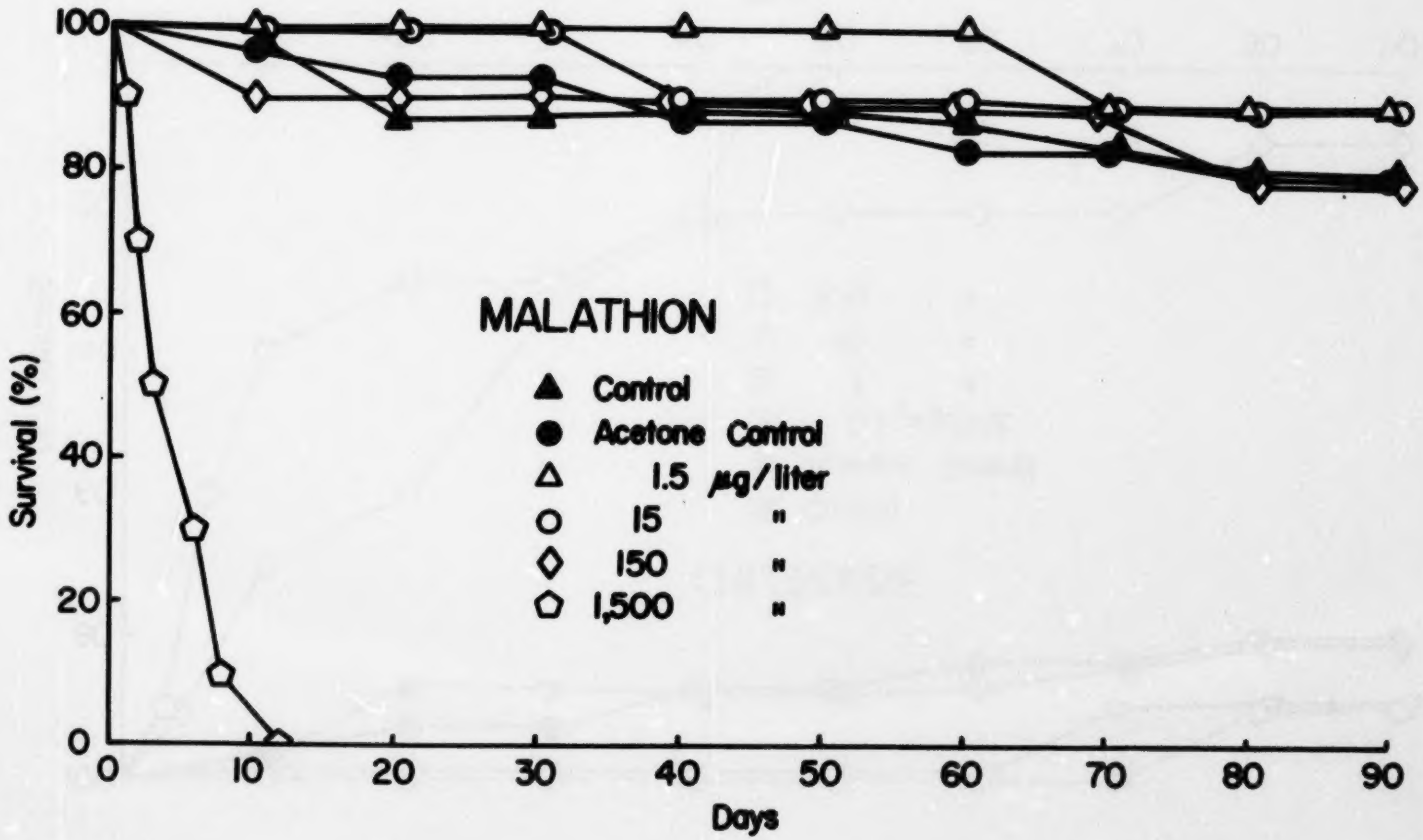


Figure 69. Effect of continuous exposure to malathion on survival of adult Dungeness crabs.

Our data, those of Sanders and Cope (1966) and Eisler (1969) for freshwater and marine crustacea, and of Sanders and Cope (1968) for insects, indicate that the toxicity of malathion for arthropods is substantially higher than for freshwater and marine fishes and marine molluscs. Macek and McAllister (1970) found that the 96-hr LC50s for 12 species of freshwater fishes ranged from a low of 101 μg/liter for coho salmon to 12,900 μg/liter for the black bullhead, Ictalurus melas. Marine fishes may be slightly more sensitive to the insecticide. Eisler (1970) found that the 96-hr LC50s for seven species of marine fishes ranged from a low of 27 μg/liter for the bluehead, Thalassoma bifasciatum, to 3,250 μg/liter for the Northern puffer, Sphaeroides maculatus. The most sensitive fish tested were fingerlings of the striped bass, Morone saxatilis for which the 96-hr LC50 was reported to be 14 μg/liter (Korn and Earnest, 1974). Molluscs are the least sensitive to malathion. Davis and Hidu (1969) found that the 48-hr LC50 for egg development of Crassostrea virginica was 9,070 μg/liter and the 14-day LC50 for larval development of the same species was 2,660 μg/liter. Clams, Merceneria merceneria, exposed to the insecticide were not killed at 37,000 μg/liter (Eisler and Weinstein, 1967), but altered metal profiles were noted in the tissues at only 37 μg/liter.

The acute toxicity of the cyclodiene insecticide chlordane to Dungeness crabs is similar to that reported for a multitude of other aquatic species representing several phyla. The 96-hr EC50 concentration for inhibition of swimming of first stage zoeae was 1.3 μg/liter of chlordane (Table 20). The chlordane 96-hr LC50 for this stage was not precisely determined since concentrations higher than 10 μg/liter were not employed, but would be expected to fall only slightly above 10 μg/liter (Table 20). With adult crabs, the 96-hr LC50 for this insecticide was 220 μg/liter (Table 20). Henderson et al. (1959), Katz (1961), Korn and Earnest (1974) and Macek et al. (1969) have found that the 96-hr LC50s for 10 species of fish are in the range of 11.8 μg/liter for striped bass, Morone saxatilis, to 190 μg/liter for the guppy, Lebistes reticulatus. The 96-hr EC50s for immobilization of the freshwater crustaceans, Simocephalus serrulatus and Daphnia pulex, are 20 and 29 μg/liter, respectively (Sanders and Cope, 1966), and for naiads of the stonefly Pteronarces californica, the 96-hr LC50 is 15 μg/liter (Sanders and Cope, 1968). The growth of young oysters, Crassostrea virginica, was adversely affected by 10 μg/liter chlordane (Butler et al.,1960).

We are unaware of studies of the acute toxicity of the carbamate insecticide carbofuran to aquatic species for which comparisons to our work can be made. Our results indicate, however, that the toxicity of this compound to the Dungeness crab is similar to that reported for another carbamate insecticide, Sevin. Buchanan et al. (1970) have reported that the 96-hr LC50 for the first instar zoeae exposed to Sevin at 10°C was 10 μg/liter and that the 48-hr LC50 at 17°C was 5 μg/liter. We have found that the carbofuran 96-hr LC50 for the first instar zoeae at 13°C is 2.5 μg/liter (Table 20). Buchanan et al. (1970) also determined the 96-hr LC50s of Sevin to adult female Dungeness crabs at 11 and 18°C, and these values were approximately 280 and 180 μg/liter, respectively. In our work with adult crabs, the 96-hr LC50 for carbofuran at 13°C was 190 μg/liter (Table 20).

Several studies have been concerned with the effects of a variety of insecticides on the larval development of crabs. These investigations, covering four major families of insecticides, the organochlorines, the organophosphates, the cyclodienes and the carbamates, are summarized in Table 21. Although there is substantial overlap in the toxicities of representatives of the four families, there is some indication that the toxicities can be ordered as follows: organochlorines = organophosphates> cyclodienes = carbamates. For all pesticides and all species of crabs, the range of concentrations, representing the lowest concentrations at which adverse effects on the larvae have been observed, is from 0.01 to 5.0 µg/liter. Our observation with methoxychlor falls within the range of toxicities reported for other organochlorine insecticides and other species. Our data for chlordane and carbofuran represent the highest toxicities reported for the cyclodiene and carbamate insecticides, respectively, and our data for malathion provide the only indication of the toxicity of the organophosphates to larval crabs.

As a result of the high toxicities of chlordane, carbofuran and malathion to larval C. magister, each of these insecticides should be considered as potentially serious contaminents of marine waters. However, with the possible exception of chlordane, these compounds are not thought to be particularly persistent in aquatic ecosystems (Liu and Lee, 1975; Sanborn, 1974) a factor which would minimize the hazard of their use near marine environments.

SUMMARY OF PESTICIDE TOLERANCE

The relative sensitivity of the life history stages of C. magister employed in this study for each of nine pesticides is summarized in Table 22. The data given are the highest no-effect concentrations derived from the particular test except in the case of zoeae. The data given for zoeae are the highest concentrations at which no adverse effect was seen on survival during chronic exposures. In some instances, to be discussed below, these concentrations were found to have an effect on the molting of larvae. Although the range of toxicity varied by more than five orders of magnitude, from the highly toxic methoxychlor to the relatively non-toxic 2,4-D acid, a uniformity of response with respect to the relative sensitivity of various life history stages is evident. The stage exhibiting the highest sensitivity to pesticides was the zoeal stage. Data were not obtained for the long-term toxicity of chlordane, malathion and carbofuran to juvenile crabs, but for the other six pesticides, the tolerance of juvenile stages was some five to ten times greater on the average than the zoeae. Propanil represented an extreme case in which juvenile stages did not appear to be more resistant than zoeal stages. At the other extreme, juvenile stages exposed to trifluralin, captan and 2,4-D acid were more than ten times as resistant as zoeal stages.

Adult crabs were also substantially more tolerant of the nine pesticides than the zoeal stages. At one extreme, adults were only five times more tolerant of propanil and DEF than the zoeal stages, and at the other extreme, adults were 750 times more tolerant of malathion than the zoeae. After summarizing the data for all nine pesticides, it would appear to be a reasonable approximation that adult stages averaged from 10 to 100 times more resistant

TABLE 21. TOXIC CONCENTRATIONS OF VARIOUS INSECTICIDES TO LARVAL CRABS

Insecticide	Species	Toxic concentration * (µg/liter)	Reference
Organochlorine			
Mirex	*Rhithropanopeus harrisii*	0.01	Bookhout et al., 1972
Mirex	*Menippe mercenaria*	0.01	Bookhout et al., 1972
Methoxychlor	*Cancer magister*	0.05	This study
Endrin	*Cancer magister*	0.25	Poole and Willis, 1970
DDT	*Cancer magister*	0.50	Poole and Willis, 1970
Mirex	*Callinectes sapidus*	1.0	Bookhout and Costlow, 1975
Organophosphate			
Malathion	*Cancer magister*	0.2	This study
Cyclodiene			
Chlordane	*Cancer magister*	0.15	This study
Dieldrin	*Leptodius floridanus*	1.0	Epifanio, 1971
Dieldrin	*Panopeus herbstii*	5.0	Epifanio, 1971
Carbamate			
Carbofuran	*Cancer magister*	0.5	This study
Sevin	*Cancer magister*	3.2	Buchanan et al., 1970

* Lowest concentration at which adverse affects seen during long-term exposures

TABLE 22. RELATIVE SENSITIVITY OF VARIOUS LIFE HISTORY STAGES OF *C. MAGISTER* TO NINE PESTICIDES. DATA ARE EXPRESSED IN µg/LITER

	Life history stage				
Pesticide	Egg *	Prezoeal +	zoeal ‡	juvenile ‡	adult ‡
Methoxychlor	>10	0.033	0.005	0.04	0.4
Chlordane	>330	<1	0.015	---	1
Malathion	>100	33	0.2	---	150
Carbofuran	>330	1	0.5	---	25
DEF	>100	33	4	15	20
Trifluralin	>330	>330	15	>150	>100
Captan	>10,000	>10,000	20	>200	>200
Propanil	>33,000	>33,000	80	80	400
2,4-D acid	100,000	33,000	1000	>10,000	>10,000

* Highest concentration at which no decrease in egg hatchability was observed during a 24-hr observation period.

+ Highest concentration at which no decrease in percentage of prezoeae molting to zoeae was observed during a 24-hr observation period.

‡ Highest concentration at which no adverse effect on survival was seen during exposure periods of at least 69 days.

to pesticides than the zoeal stages.

The toxicity data for the egg and prezoeal stages are not as readily compared to those for the zoeae because these stages were tested during extremely short periods of time, and because the criteria of effect were different. The prezoeal stage is of extremely short duration, from 10 to 60 min at the temperatures tested (Buchanan and Millemann, 1969), and represents the brief period in the life history between the hatching of the crab eggs and the first zoeal stage. The criterion of effect employed for prezoeae was failure to molt to the zoeal stage, which, considering the 24-hr observation period, could be considered a lethal effect. By this criterion, the tolerance of the prezoeal stage relative to that of the zoeae ranged from twice for carbofuran to >412 and >500 for propanil and captan, respectively (Table 22.)

In all probability, the greater apparent tolerance of prezoeae is attributable to the very short duration of this stage, and, therefore, the short duration of pesticide exposure, relative to that of the first through fifth zoeae. If the periods of exposure were the same, the prezoeae would likely be about as sensitive to pesticides as first stage zoeae. This is indicated by the ratios obtained for each pesticide of the 24-hr LC50s for zoeae to the "no effect" levels for prezoeae. These ratios range from <0.04 for malathion to 27.9 for methoxychlor. Since the actual exposure period for prezoeae is undoubtedly much less than 24 hr, these ratios are probably conservatively low. Therefore, where only short-term contamination of marine waters by pesticides is expected, effects on the prezoeae may be as important or more important than effects on zoeae, especially for some compounds. Nevertheless, it is evident that under conditions of chronic pesticide exposure, the prezoeal stage should not be considered as a sensitive stage in the life history, since at concentrations below those that affect prezoeae, the zoeal stage is still likely to be affected (Table 22).

The eggs, as judged by egg hatchability, appear to be even less affected by pesticide exposures on the average than the prezoeal stage (Table 22). With the exception of 2,4-D acid, egg hatchability was not adversely affected by any of the pesticides examined at the highest concentrations - - concentrations which greatly exceeded those that affected zoeae in the chronic exposure tests. In the egg hatching tests, the highest concentrations of DEF and trifluralin were 100 and 330 μg/liter, and exceeded the no-effect concentrations in the zoeal tests by 25 and 22 times, respectively. Furthermore, no-effect concentrations for these two pesticides and eggs could have been substantially higher. At the other extreme, the highest concentration of chlordane in the egg tests, 330 μg/liter, a concentration which had no effect, was 22,000 times greater than the highest no-effect level of chlordane in the zoeal tests. Only with 2,4-D acid was an adverse effect observed on egg hatching, but the no-effect level for this response was 100,000 μg/liter, a concentration exceeding the no-effect level against first stage zoeae by 100 times. These data suggest that eggs are not a particularly sensitive stage of the life history of crabs with respect to pesticide exposure. This observation, however, should not be considered conclusive until much more extensive tests with eggs are conducted. The experiments reported here involved exposure of eggs only just prior to hatching and for a very short period of time. Exposures of eggs to pesticides earlier in their developmental sequence and for longer periods of time might result in different conclusions.

The objective of the studies described in this report was to determine for the Dungeness crab, Cancer magister, the maximum concentration of each pesticide that could be tolerated by the most sensitive stage in the life history of the crab. Since the time required for this species to complete the life cycle is about 3 to 4 years (Butler, 1961), it was not feasible to estimate the maximum acceptable toxicant concentration as recommended by Mount and Stephen (1967). Our approach, therefore, was to expose distinct stages in the life history of the crab to uniform concentrations of each pesticide for periods of up to two to three months and determine for each such stage the maximum tolerable concentration of each compound by using survival and appropriate sublethal criteria to judge effects.

We have shown for each pesticide that the zoeal stages during chronic exposures represented the most sensitive stages for the species (Table 22). In addition, we have found in the majority of cases that survival was as sensitive a criterion of effect for this stage as molt inhibition, the sublethal effect employed. For methoxychlor, chlordane, trifluralin, propanil and DEF, the highest concentration that had no effect on survival also had no apparent effect on larval molting. In the case of methoxychlor, the threshold concentration for effects on survival and molting appeared to be approximately the same. Larvae exposed to 0.5 µg/liter were rapidly killed (Figure 14); those surviving the time of the first molt also experienced a significant delay in molting (Figure 15). Larvae exposed to 0.05 µg/liter of methoxychlor exhibited a slightly decreased survival compared to controls and also appeared to molt slightly later than the control zoeae. Those exposed to 0.005 µg/liter experienced neither a decrease in survival nor a delay in molting.

For larvae exposed chronically to captan and 2,4-D, and possibly also for those exposed to malathion and carbofuran, molting was a slightly more sensitive response to the pesticide exposure than survival. The survival of larvae was not affected during 69 days of exposure to 20 µg/liter of captan (Figure 6), but this concentration resulted in a delay in molting of about 3 to 4 days during the third and fourth zoeal molts (Figure 7). Even at 2 µg/liter, captan delayed the time of the third molt, but this delay was no longer apparent by the time of the fourth molt and may represent an anomolous event. A concentration of 10,000 µg/liter of 2,4-D acid significantly affected survival of developing crab larvae (Figure 36) and also substantially delayed their molting (Figure 41). This herbicide had no effect on survival of larvae at 1,000 µg/liter, but molting was still delayed. Larvae exposed to this concentration of 2,4-D molted about 2 to 3 days later than control larvae during each molt, indicating that the effect on molting occurred at the time of the first molt; i.e., the duration of second, third and fourth zoeal stages was not actually longer than these stages in the control larvae. Since a lower concentration of 2,4-D was not employed in these tests, the threshold concentration for the effect on molting could not be ascertained, but is presumed to fall only moderately below 1,000 µg/liter.

The data for carbofuran and malathion are equivocal and, as for methoxychlor, the threshold of effects on survival and molt delay may be very similar. The highest no-effect concentration of carbofuran for survival was 0.5 µg/liter (Figure 61), but at this concentration the larvae appeared to be molting slightly later than the control larvae (Figure 62). A similar relationship was observed for the insecticide malathion at an exposure concentration of 0.2 µg/liter. For this pesticide, a delay in molting was not at all apparent until the time of the fourth molt, and the results are only suggestive of a delay even at that time (Figure 64).

No other sublethal effects were systematically examined in larval crabs during the chronic exposure experiments. However, we did not, by casual observation, detect any effects on swimming activity, positioning behavior or size. The only other sublethal effects studied were those relating to osmotic and ionic regulation in adult crabs and the size of molted juvenile crabs exposed to methoxychlor. In the former experiments, crabs were exposed for up to 14 days to 10 µg/liter of the pesticide, a concentration which should have resulted in 50% mortality upon continuous exposures for 30 to 60 days (Figure 19). Under these exposure conditions, an effect was noted on salinity tolerance (Table 17), gill NaKMgATPase activity (Table 8) and blood/urine Mg^{++} regulation (Figure 25), but not on regulation of Na^+ or K^+ ions or on blood osmotic pressure (Figures 22 to 24). Juvenile crabs exposed to 4.0 µg/liter of methoxychlor were smaller than controls following the molt to fourth instar, but this effect also occurred at a concentration of the insecticide that was eventually lethal (Figure 17). These studies with older crabs also suggest that sublethal effects are only marginally apparent except at very nearly lethal concentrations. In addition, these sublethal effects on adult and juvenile crabs occurred at concentrations substantially higher than those causing impaired survival of larvae during chronic exposure.

We believe, therefore, that it is reasonable to conclude that the maximum acceptable toxicant concentrations for the nine pesticides studied against the Dungeness crab, *Cancer magister*, are equivalent to the highest concentrations which do not affect larval survival during chronic exposures for methoxychlor, chlordane, trifluralin, propanil, and DEF, and are the highest concentrations which do not affect larval molting for the pesticides malathion, carbofuran, captan, and 2,4-D acid. These concentrations are listed in Table 23. Although we believe that these represent "safe" concentrations for long-term exposures of *C. magister* to each pesticide, individually, it should be reemphasized that they are not strictly equivalent to the maximum acceptable toxicant concentrations as defined by Mount and Stephen (1967). These workers defined the maximum acceptable toxicant concentration as that concentration which during continuous exposure throughout an entire life cycle does not affect any aspect of the biology of the organism. It is necessary to reemphasize that we have not been able to extend our tests for the full 3- to 4-year period necessary to achieve this result, and we cannot be certain that the concentrations given in Table 22 would have no effect on reproductive processes of the organism. We believe, however, that they may be applied as criteria for the protection of this species with reasonable assurance.

TABLE 23. ESTIMATED MAXIMUM ACCEPTABLE TOXICANT CONCENTRATIONS (MATC)* FOR CONTINUOUS EXPOSURE OF CRABS TO EACH OF NINE PESTICIDES

Pesticide	Estimated maximum acceptable toxicant concentration (MATC) for continuous exposure (μg/liter)	Stage and criteria employed for estimation of MATC
Methoxychlor	0.005	zoeal survival (Figure 14)
Chlordane	0.015	zoeal survival (Figure 65)
Malathion	0.02	zoeal molting (Figure 64)
Carbofuran	0.05	zoeal molting (Figure 62)
Captan	2	zoeal molting (Figure 7)
DEF	4	zoeal survival (Figure 37)
Trifluralin	15	zoeal survival (Figure 34)
Propanil	80	zoeal survival (Figure 35)
2,4-D acid	1000	zoeal molting (Figure 41)

* Maximum acceptable toxicant concentration (MATC) is defined as the highest concentration of each pesticide tested individually at which no lethal or sublethal effects were observed in any acute or chronic tests in this study. It does not represent the highest no effect concentration for continuous exposure throughout an entire 3- to 4-year life history cycle and, therefore, may not adequately define the "safe" level for reproductive processes other than larval development and egg hatching.

REFERENCES

Abedi, Z. H. and W. P. McKinley. 1967. Bioassay of Captan by Zebra-fish Larvae. Nature 216:1321-1322.

Alspach, G. S. 1972. Osmotic and Ionic Regulation in the Dungeness Crab, Cancer magister (Dana). Ph.D. Dissertation, Oregon State University, Corvallis, Oregon. 86 pp.

American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1971. Standard Methods for the Examination of Water and Wastewater. 13th ed. Washington, D.C. 874 pp.

Bookhout, C. G. and J. D. Costlow, Jr. 1975. Effects of Mirex on the Larval Development of Blue Crab. Water Air Soil Pollut. 4:113-126.

Bookhout, C. G., A. J. Wilson, Jr., T. W. Duke and J. L. Lowe. 1972. Effects of Mirex on the Larval Development of Two Crabs. Water Air Soil Pollut. 1:165-180.

Buchanan, D. V. and R. E. Millemann. 1969. The Prezoeal Stage of the Dungeness Crab, Cancer magister Dana. Biol. Bull. 137:250-255.

Buchanan, D. V., R. E. Millemann and N. E. Stewart. 1970. Effects of the Insecticide Sevin on Various Stages of the Dungeness Crab, Cancer magister. J. Fish. Res. Bd. Canada 27:93-104.

Butler, P. A. 1965. Effects of Herbicides on Estuarine Fauna. Proc. South Weed Conf. 18:576-580.

Butler, P. A., A. J. Wilson, Jr. and A. J. Rick. 1960. Effect of Pesticides on Oysters. Proc. Natl. Shellfish. Assoc. 51:23-32.

Butler, T. H. 1961. Growth and Age Determination of the Pacific Edible Crab Cancer magister Dana. J. Fish. Res. Bd. Canada 18:873-891.

Chance, B. and B. Hess. 1959. Metabolic Control Mechanisms. I. Electron Transfer in the Mammalian Cell. J. Biol. Chem. 234:2404-2412.

Cope, O. B. 1965. Some Responses of Freshwater Fish to Herbicides. Proc. South Weed Conf. 18:439-445.

Costlow, J. D., Jr. 1966. The Effect of Eyestalk Extirpation on Larval Development of the Mud Crab, Rithropanopeus harrisii (Gould). Gen. Comp. Endocr. 7:255-274.

Crosby, D. G. and R. K. Tucker. 1966. Toxicity of Aquatic Herbicides to *Daphnia magna*. Science 154:289-291.

Davis, H. C. and H. Hidu. 1969. Effects of Pesticides on Embryonic Development of Clams and Oysters and on Survival and Growth of the Larvae. U.S. Fish. Wildl. Serv. Fish. Bull. 67:383-404.

Davis, P. W., J. M. Friedhoff and G. A. Wedemeyer. 1972. Organochlorine Insecticide, Herbicide, and Polychlorinated Biphenyl (PCB) Inhibition of NaK-ATPase in Rainbow Trout. Bull. Environ. Contam. Toxicol. 8:69-72.

Dehnel, P. A. 1962. Aspects of Osmoregulation in Two Species of Intertidal Crabs. Biol. Bull. 122:208-227.

Dehnel, P. A. and T. H. Carefoot. 1965. Ion Regulation in Two Species of Intertidal Crabs. Comp. Biochem. Physiol. 15:377-397.

Duke, T. W., J. I. Lowe and A. J. Wilson, Jr. 1970. A Polychlorinated Biphenyl (Aroclor 1254®) in the Water, Sediment, and Biota of Escambia Bay, Florida. Bull. Envir. Contam. Toxicol. 5:171-180.

Eisler, R. 1969. Acute Toxicities of Insecticides to Marine Decapod Crustaceans. Crustaceana 16:302-310.

Eisler, R. 1970. Acute Toxicities of Organochlorine and Organophosphorus Insecticides to Estuarine Fishes. Bureau of Sport Fisheries and Wildlife Tech. Paper 46. Gov. Printing Office, Washington, D.C. 12 pp.

Eisler, R. and M. P. Weinstein. 1967. Changes in Metal Composition of the Quahaug Clam, *Mercenaria mercenaria*, after Exposure to Insecticides. Chesapeake Sci. 8:253-258.

Engelhardt, F. R. and P. A. Dehnel. 1973. Ionic Regulation in The Pacific Edible Crab, *Cancer magister* (Dana). Can. J. Zool. 51:735-743.

Epifanio, C. E. 1971. Effects of Dieldrin in Seawater on the Development of Two Species of Crab Larvae, *Leptodius floridanus* and *Panopeus herbstii*. Mar. Biol. 11:356-362.

Fabro, S., R. L. Smith and R. T. Williams. 1965. Embryotoxic Activity of Some Pesticides and Drugs Related to Phthalimide. Food Cosmet. Toxicol. 3:587-590.

Fiske, C. H. and Y. SubbaRow. 1925. The Colorimetric Determination of Phosphorus. J. Biol. Chem. 66:375-400.

Frear, D. E. H. and J. E. Boyd. 1967. Use of *Daphnia magna* for the Microbioassay of Pesticides. I. Development of Standardized Techniques for Rearing *Daphnia* and Preparation of Dosage-Mortality Curves for Pesticides. J. Econ. Entomol. 60:1228-1236.

Gross, W. J. 1964. Trends in Water and Salt Regulation among Aquatic and Amphibious Crabs. Biol. Bull. 127:447-466.

Gross, W. J. and R. L. Capen. 1966. Some Functions of the Urinary Bladder in a Crab. Biol. Bull. 131:272-291.

Gross, W. J. and L. A. Marshall. 1960. The Influence of Salinity on the Magnesium and Water Fluxes of a Crab. Biol. Bull. 119:440-453.

Henderson, C., Q. H. Pickering and C. M. Tarzwell. 1959. Relative Toxicity of Ten Chlorinated Hydrocarbon Insecticides to Four Species of Fish. Trans. Am. Fish. Soc. 88:23-32.

Hermanutz, R. O., L. H. Mueller, and K. D. Kempfert. 1973. Captan Toxicity to Fathead Minnows (Pimephales promelas), Bluegills (Lepomis machrochirus), and Brook Trout (Salvelinus fontinalis). J. Fish. Res. Bd. Can. 30:1811-1817.

Holden, E. R. 1973. Determination of Residues of Methylcarbamate Insecticides in Crops by Gas Chromatography of their 2,4-Dinitrophenyl Ether Derivatives. J. Assoc. Off. Anal. Chem. 56:713-717.

Holland, G. A., J. E. Lasater, E. D. Neumann and W. E. Eldridge. 1960. Toxic Effects of Organic and Inorganic Pollutants on Young Salmon and Trout. Wash. Dept. U.S. Fish. Wildl. Serv. Fish. Bull. No. 5.

Hughes, J. S. and J. T. Davis. 1963. Variations in Toxicity to Bluegill Sunfish of Phenoxy Herbicides. Weeds 11:50-53.

Johnson, B. T. and J. O. Kennedy. 1973. Biomagnification of p,p'-DDT and Methoxychlor by Bacteria. Appl. Microbiol. 26:66-71.

Kapoor, I. P., R. L. Metcalf, R. F. Nystron and G. K. Sangha. 1970. Comparative Metabolism of Methoxychlor, Methiochlor, and DDT in Mouse, Insects, and in a Model Ecosystem. J. Agric.Food Chem. 18:1145-1152.

Katz, M. 1961. Acute Toxicity of Some Organic Insecticides to three species of Salmonids and to the Threespine Stickleback. Trans. Am. Fish. Soc. 90:264-268.

Kennedy, G., O. E. Fancher and J. C. Calandra. 1968. An Investigation of the Teratogenic Potential of Captan, Folpet, and Difolatan. Toxicol. Appl. Pharmacol. 13:420-430.

Kinter, W. B., L. S. Merkens, R. H. Janicki and A. M. Guarino. 1972. Studies on the Mechanism of Toxicity of DDT and Polychlorinated Biphenyls (PCBs): Disruption of Osmoregulation in Marine Fish. Environ. Health Perspect. 1:169-173.

Korn, S. and R. Earnest. 1974. Acute Toxicity of 20 Insecticides to a Striped Bass, Morone saxatilis. Calif. Fish Game 60:128-131.

Legator, M. S., F. J. Kelly, S. Green and E. J. Oswald. 1969. Mutagenic Effects of Captan. Ann. N.Y. Acad. Sci. 160:344-351.

Liu, D. H. W. and J. M. Lee. 1975. Toxicity of Selected Pesticides to the Bay Mussel (Mytilus edulis). EPA-660/3-75-016, U.S. Environmental Protection Agency, Corvallis, OR. 102 pp.

Lockwood, A. P. M. 1962. The Osmoregulation of Crustacea. Biol. Rev. 37:257-305.

Lockwood, A. P. M. 1967. Aspects of the Physiology of Crustacea. W. H. Freeman, San Francisco, CA. 328 pp.

Lockwood, A. P. M. and J. A. Riegel. 1969. The Excretion of Magnesium by Carcinus maenas. J. Exp. Biol. 51:575-589.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193:265-275.

Macek, K. J. and W. A. McAllister. 1970. Insecticide Susceptibility of Some Common Fish Family Representatives. Trans. Am. Fish. Soc. 99:20-27.

Macek, K. J., C. Hutchinson and O. B. Cope. 1969. The Effects of Temperature on the Susceptibility of Bluegills and Rainbow Trout to Selected Pesticides. Bull. Environ. Contam. Toxicol. 4:174-183.

Matsumura, F. and T. Narahashi. 1971. ATPase Inhibition and Electrophysiological Change Caused by DDT and Related Neuroactive Agents in Lobster Nerve. Biochem. Pharmacol. 20:825-837.

Matsumura, F. and K. C. Patil. 1969. Adenosine Triphosphatase Sensitive to DDT in Synapses of Rat Brain. Science 166:121-122.

McLaughlin, J., Jr., E. F. Reynaldo, J. K. Lamar and J.-P. Marliac. 1969. Teratology Studies in Rabbits with Captan, Folpet, and Thalidomide. Toxic. Appl. Pharmacol. 14:641.

Meehan, W. R., L. A. Norris and H. S. Sears. 1974. Toxicity of Various Formulations of 2,4-D to Salmonids in Southeast Alaska. J. Fish. Res. Bd. Can. 31:480-485.

Mount, D. I. and C. E. Stephen. 1967. A Method for Establishing Acceptable Toxicant Limits for Fish. Malathion and the Butoxy-Ethanol Ester of 2,4-D. Trans. Am. Fish. Soc. 96:185-193.

Narahashi, T. and H. G. Hass. 1967. DDT: Interaction with Nerve Membrane Conductance Changes. Science 157:1438-1440.

Nelson, B. D. 1971a. Action of the Fungicides Captan and Folpet on Rat Liver Mitochondria. Biochem. Pharmacol. 20:737-748.

Nelson, B. D. 1971b. Induction of Mitochondrial Swelling by the Fungicide Captan. Biochem. Pharmacol. 20:749-758.

Nimmo, D. R., A. J. Wilson, Jr. and R. R. Blackman. 1970. Localization of DDT in the Body Organs of Pink and White Shrimp. Bull. Envir. Contam. Toxicol. 5:333-341.

Nimmo, D. R., R. R. Blackman, A. J. Wilson, Jr. and J. Forester. 1971. Toxicity and Distribution of Aroclor® 1254 in the Pink Shrimp *Penaeus duorarum*. Mar. Biol. 11:191-197.

O'Brien, R. D. 1967. Insecticides: Action and Metabolism. Academic Press, New York. 332 pp.

Owens, R. G. and G. Blaak. 1960a. Site of Action of Captan and Dichlone in the Pathway Between Acetate and Citrate in Fungus Spores. Contr. Boyce Thomson Inst. Pl. Res. 20:459-474.

Owens, R. G. and G. Blaak. 1960b. Chemistry of the Reactions of Dichlone and Captan with Thiols. Contr. Boyce Thomson Inst. Pl. Res. 20:475-497.

Oxender, D. L. 1972. Membrane Transport. Annu. Rev. Biochem. 41:777-809.

Parka, S. J. and H. M. Worth. 1965. The Effects of Trifluralin on Fish. Proc. South Weed Conf. 18:469-473.

Passano, L. M. 1960 Molting and Its Control. pp. 473-536. In: The Physiology of Crustacea. Vol. 1. Metabolism and Growth. (Waterman, T. H., Ed.) Academic Press, New York.

Poole, R. L. and M. Willis. 1970. Effects of Some Pesticides on Larvae of the Market Crab, *Cancer magister*, and the Red Crab, *Cancer productus*, and a Bioassay of Industrial Wastes with Crab Larvae. Manuscript report, California Dept. Fish and Game, Marine Resources Region, reference No. 70-15, 19 pp.

Reinbold, K. A., I. P. Kapoor, W. F. Childers, W. N. Bruce and R. L. Metcalf. 1971. Comparative Uptake and Biodegradability of DDT and Methoxychlor by Aquatic Organisms. Ill. State Nat. Hist. Surv. Bull. 30:405-415.

Richmond, D. V. and E. Somers. 1966. Studies on the Fungitoxicity of Captan. IV. Reactions of Captan with Cell Thiols. Ann. Appl. Biol. 57:231-240.

Sanborn, J. R. 1974. The Fate of Selected Pesticides in the Aquatic Environment. EPA-660/3-74-025. U.S. Environmental Protection Agency, Corvallis, OR. 83 pp.

Sanders, H. O. 1969. Toxicity of Pesticides to the Crustacean, *Gammarus lacustris*. U.S. Bur. Sport Wildl. Tech. Pap. 25. Government Printing Office, Wash. D.C., 18 pp.

Sanders, H. O. 1970. Toxicities of Some Herbicides to Six Species of Freshwater Crustaceans. J. Water Poll. Control Fed. 42:1544-1550.

Sanders, H. O. and O. B. Cope. 1966. Toxicities of Several Pesticides to Two Species of Cladocerans. Trans. Am. Fish. Soc. 95:165-169.

Sanders, H. O. and O. B. Cope. 1968. The Relative Toxicities of Several Pesticides to Naiads of Three Species of Stoneflies. Limnol. Oceanog. 13:112-117.

Schatzmann, H. J. and F. F. Vincenzi. 1969. Calcium Movements Across the Membrane of Human Red Cells. J. Physiol. 201:369-395.

Verrett, M. J., M. K. Mutchler, W. F. Scott, E. F. Reynaldo and J. McLaughlin. 1969. Teratogenic Effects of Captan and Related Compounds in the Developing Chicken Embryo. Ann. N.Y. Acad. Sci. 160:334-343.

Walsh, G. E. 1972. Effects of Herbicides on Photosynthesis and Growth of Marine Unicellular Algae. Hyacinth Control J. 10:45-48.

Wildish, D. J. and V. Zitko. 1971. Uptake of Polychlorinated Biphenyls from Seawater by Gammarus oceanicus. Mar. Biol. 9:213-218.

Yeager, J. F. and S. C. Munson. 1945. Physiological Evidence of a site of Action of DDT in an Insect. Science 102:305-307.

PUBLICATIONS

Armstrong, D. A., D. V. Buchanan, and R. S. Caldwell. 1976. A Mycosis caused by *Lagenidium* sp. in Laboratory-Reared Larvae of the Dungeness Crab, *Cancer magister*, and Possible Chemical Treatments. J. Invert. Pathol. 28:329-336.

Armstrong, D. A., D. V. Buchanan, M. H. Mallon, R. S. Caldwell, and R. E. Millemann. 1976. Toxicity of the Insecticide Methoxychlor to the Dungeness Crab, *Cancer magister*. Mar. Biol. 38:239-252.

Buchanan, D. V., M. J. Myers, and R. S. Caldwell. 1975. Improved Flowing Water Apparatus for the Culture of Brachyuran Crab Larvae. J. Fish. Res. Bd. Canada 32:1880-1883.

Caldwell, R. S. 1974. Osmotic and Ionic Regulation in Decapod Crustacea Exposed to Methoxychlor. pp 197-223. In: Pollution and Physiology of Marine Organisms (Vernberg, F. J. and W. B. Vernberg, Eds.). Academic Press, N.Y.

Caldwell, R. S., D. A. Armstrong, D. V. Buchanan, M. H. Mallon, and R. E. Millemann. 1977. Toxicity of the Fungicide Captan to the Dungeness Crab, *Cancer magister* Dana. Mar. Biol. (Submitted).

Caldwell, R. S., D. V. Buchanan, D. A. Armstrong, M. H. Mallon and R. E. Millemann. 1977. Toxicity of the Herbicides 2,4-D, DEF, Propanil and Trifluralin to the Dungeness Crab, *Cancer magister*. (In preparation).

Caldwell, R. S., D. V. Buchanan, D. A. Armstrong, M. H. Mallon, and R. E. Millemann. 1977. Toxicity of the Insecticides Carbofuran, Chlordane and Malathion to the Dungeness Crab, *Cancer magister*. (In preparation).

* U.S. GOVERNMENT PRINTING OFFICE: 1978--740-263/1525 Region No. 4